imb

Institute of
Molecular Biology

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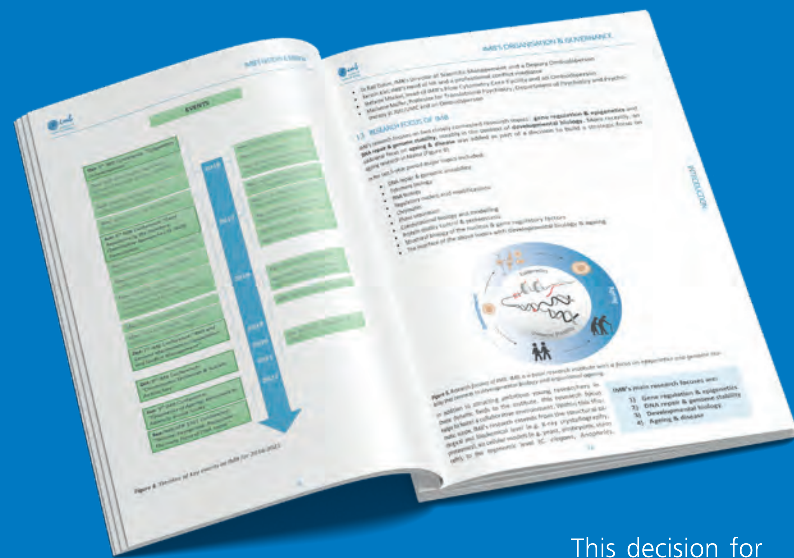
FOREWORD

The year 2021 ended on three high notes for IMB. First, IMB underwent its 10-year institutional review, in which we were evaluated by a panel of 22 international researchers over 2.5 days. We are extremely pleased that the reviewers considered IMB an 'excellent' research institution, reassuring us that we are on the right path and providing an important step to securing continued funding. Second, IMB's International PhD Program (IPP) – which encompasses 173 students from 45 countries – was also evaluated and received the highest praise from reviewers. Third, news broke that the new German Federal Government plans to found a Helmholtz Centre for Ageing Research in Mainz, which will provide vast new opportunities for IMB and the Mainz campus at large.

FOREWORD



This decision for a Mainz Helmholtz Centre for Ageing Research echoes IMB's efforts to expand its research focus on cellular and organismic ageing, which we have already been working towards for several years. In connection with this goal, IMB recently established the virtual Centre of Healthy Ageing (CHA), which brings together 41 research groups across Mainz with an interest in ageing research. In addition, IMB spearheaded the SHARP (Science of Healthy Ageing Research Programme) initiative, which was recently funded by Rhineland-Palatinate's Ministry of Science and Health. SHARP is a joint PhD training programme between IMB and Mainz's University Medical Center to promote collaborative projects bridging clinical and basic research. In this context, we were especially honoured by a visit from Olaf Scholz, who is now the chancellor of Germany, and Malu Dreyer (Prime Minister of Rhineland-Palatinate) in July, where they informed themselves of the ageing research being carried out at IMB.

The challenges of the COVID-19 pandemic have followed us into 2021, but IMB has fortunately managed to avoid any major outbreaks even as many of us worked on-site, thanks to our Health and Safety team establishing an in-house PCR test to detect infections early. Although this prevented 10-year anniversary celebrations, IMB has now adjusted well to virtual events and held numerous online lectures, seminars, meetings, workshops, and even large events such as an IPP retreat and a PhD student symposium with 128 participants.

We welcomed three new group leaders in 2021: Dorothee Dormann, Nard Kubben and Lukas Stelzl. Dorothee is an Adjunct Director at IMB with a joint appointment at Mainz University who focuses on understanding the mechanisms of protein mislocalisation and aggregation in neurodegenerative disease. Nard is a Junior Group Leader who uses a genetically engineered cell system to model human ageing and the premature ageing disease Hutchinson-Gilford progeria. Lukas is an Adjunct Junior Group Leader with an appointment at Mainz University who employs computational modelling to understand the molecular interactions that underlie phase separation of disordered proteins.

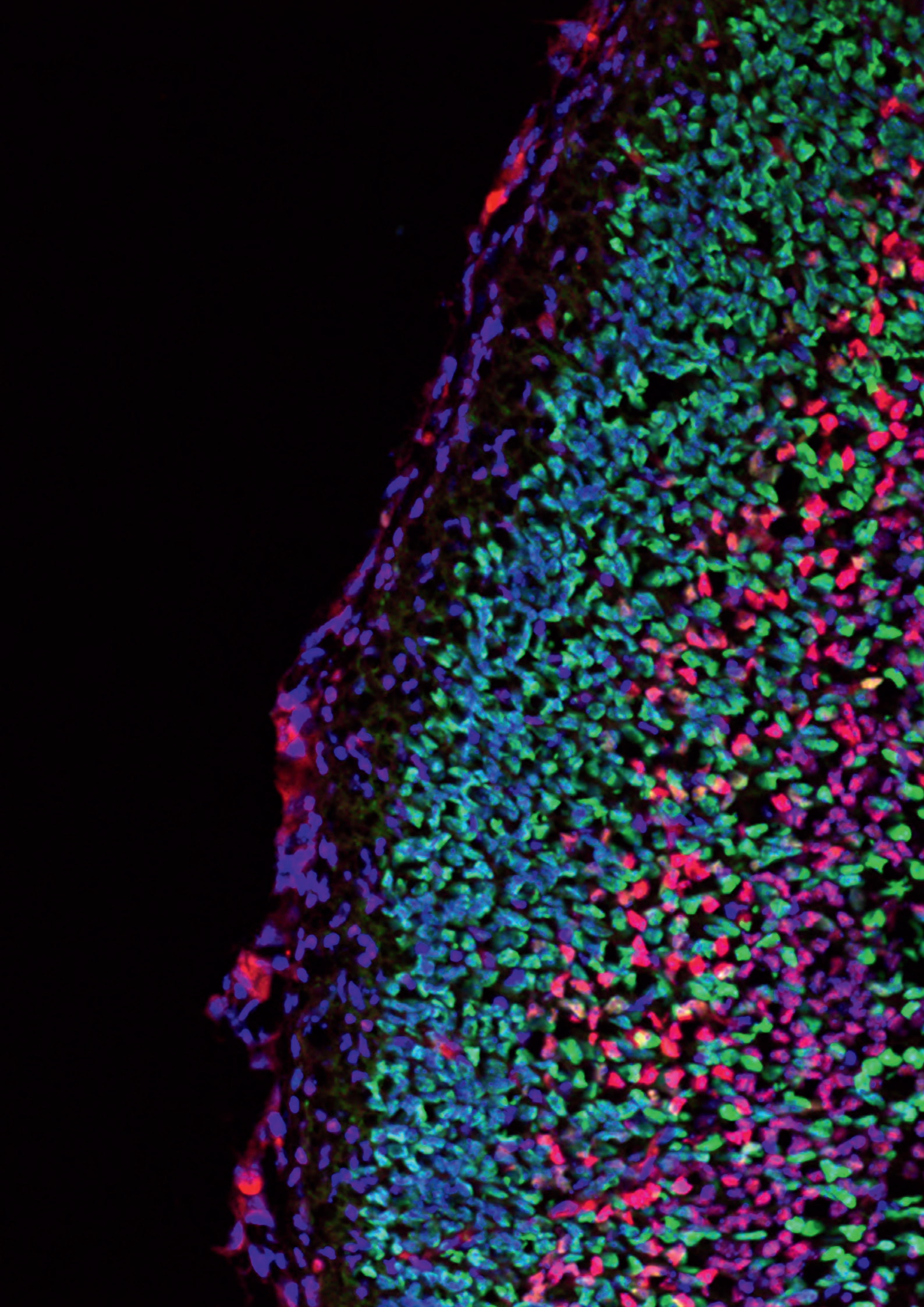
IMB's researchers produced 72 publications in 2021. A highlight was Edward Lemke's publication in *Cell*, in which his team created film-like artificial membraneless organelles that can be used to engineer multiple new functions in cells. The group of Anton Khmelinskii also published a paper in *Molecular Cell*, in which they used tandem fluorescent timers to systematically investigate the functions of proteins in the ubiquitin-protease system. In addition, the labs of René Ketting and Falk Butter described in *Nature Communications* their discovery of two previously unknown telomere-binding proteins in *C. elegans*.

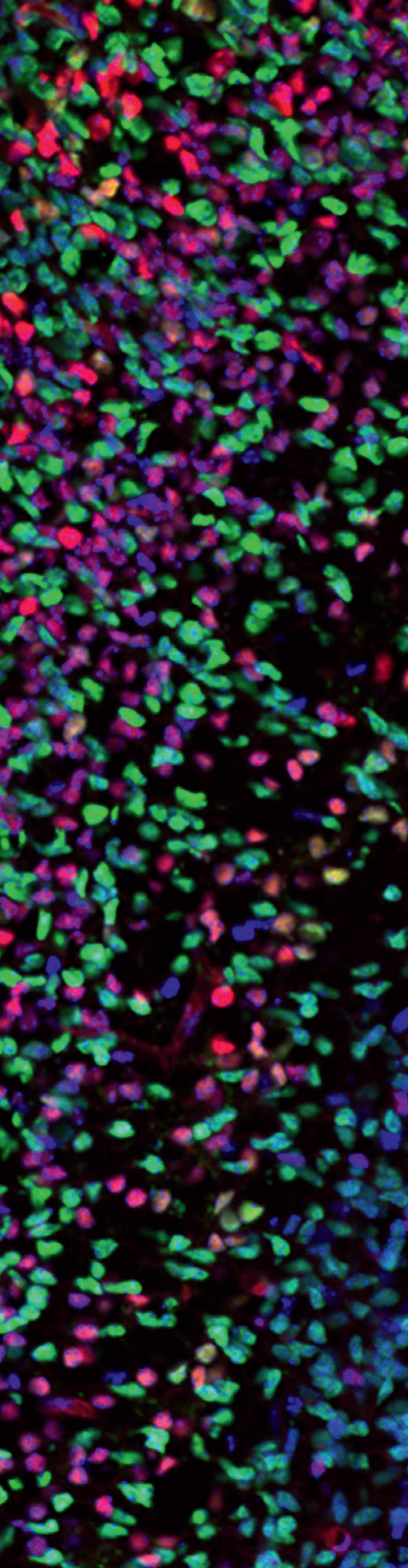
To top off these achievements, IMB's group leaders collectively raised more than €4.4 million in research funding in 2021. Edward Lemke was awarded a prestigious "Life?" grant worth €1 million from the Volkswagen Foundation for his research using membraneless organelles to investigate fundamental processes that are essential for life, and Dorothee Dormann was awarded the Alzheimer Research Award 2021 from the Hans and Ilse Breuer Foundation (worth €280,000). Finally, Helle Ulrich was elected to the prestigious German National Academy of Sciences Leopoldina, which provides policymakers and the public with science-based evidence.

Next year, we look forward to resuming our IMB Conference series with a conference on the "Epigenetics of Ageing: Responses to Adversity across Scales" in June and an IMB/CRC 1361 conference on "Restore, Reorganise, Repurpose: the Many Faces of DNA Repair" in September.

I would like to thank the Boehringer Ingelheim Foundation and the State of Rhineland-Palatinate for their support and generous funding, and the members of our Scientific Advisory Board, whose advice and feedback have been instrumental in developing IMB. I also thank my colleague René Ketting, who will take over IMB's rotating Executive Directorship in 2022. Last but certainly not least, I would like to thank all colleagues at IMB for their contributions to a spectacular outcome in IMB's 10-year review and continuing to make IMB a leader for research in the life sciences.

Christof Niehrs
Executive Director





RESEARCH GROUPS

08 BARAU

10 BAUMANN

12 BELI

14 BUTTER

16 DORMANN

18 KELLER VALSECCHI

20 KETTING

22 KHMELINSKII

24 KÖNIG

26 KUBBEN

28 LEMKE

30 LUCK

32 LUKE

34 NIEHRS

36 ROUKOS

38 SCHICK

40 STELZL

42 ULRICH

44 WOLF

JOAN **BARAU**

OVERVIEW

Transposable elements, or TEs, are abundant genomic repeats linked to genome instability and regulatory perturbations that can lead to phenotypic consequences. In addition, TE-encoded proteins can be co-opted into functional components of our genomes, and their genomic sequences into elements that instruct genomic regulation. Our lab's work focuses on understanding transposon biology as a proxy to uncover new mechanisms that affect gene regulation, genome stability and inheritance. In the past year, our lab has been working on three fronts aimed at discovering (i) how transposons are targeted for epigenetic silencing in mouse germ cells, (ii) how transposon sequences and their epigenetic status impact their regulatory potential in mouse germ cells, and (iii) novel regulators of the transposon 'life cycle' in pluripotent and differentiated stages of mammalian development.

We study transposable elements to learn more about genome regulation

RESEARCH HIGHLIGHTS

How transposons are targeted for epigenetic silencing in mouse germ cells

Germ cells have the demanding task of distinguishing 'normal' functioning genes from active TEs, which should be inactivated. This is achieved by processing TE mRNAs into small pIWI-interacting RNAs (piRNAs). Production of piRNAs allows germ cells to specifically degrade TE mRNA and guide nuclear silencing factors to active TE loci, which leads to stable, life-long epigenetic silencing by DNA methylation. We now know that the final, stable step of TE transcriptional silencing in germ cells depends on the epigenetic modifier DNMT3C. In the past year, we have experimentally connected DNMT3C with the core components of the piRNA pathway. We are now focusing on understanding the mechanisms of piRNA-guided DNA methylation and how chromatin modifications and nuclear organisation impact this process.

How transposon sequences and their epigenetic status impact their regulatory potential in mouse germ cells

Transposons live dual lives in mammalian genomes: an individual transposon can act as a developmentally important enhancer of gene expression, but it can also promote its own transposition, with potentially dire consequences for genome stability. We hypothesise that DNA methylation at TE promoters can tip the scale towards either of these opposing roles by impacting the binding of regulatory factors and interactions with readers and writers of chromatin modifications. In the past year, we learned a lot about DNA-binding factors that may

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Since 2019
Group Leader, Institute of Molecular Biology (IMB), Mainz

2013 – 2019
Postdoc, Institut Curie, Paris

EDUCATION

2012
PhD in Genetics and Molecular Biology, University of Campinas

2005
BS in Biology, University of Campinas

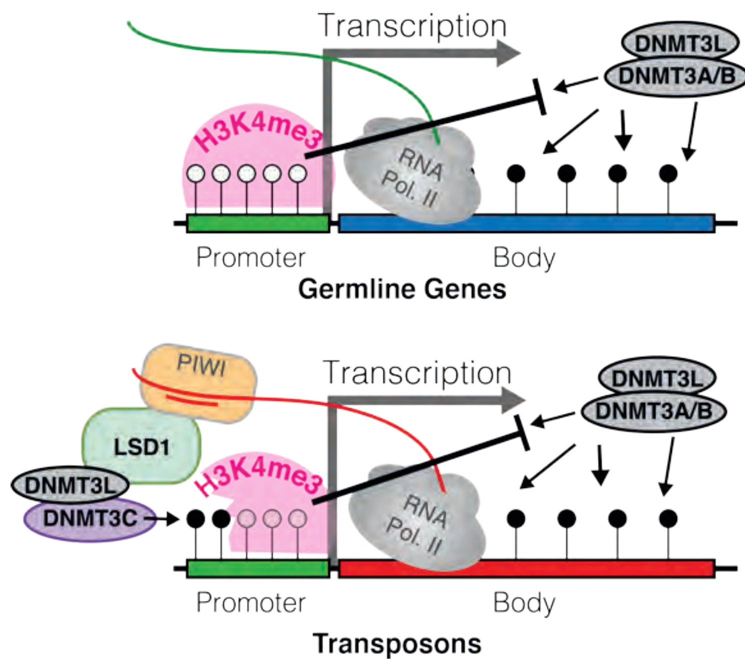

Figure 1. A model for piRNA-guided DNA methylation. *De novo* methylation of active promoters is prevented due to the repelling effect H3K4me3 has on DNA methyltransferases. TE promoters are recognised by MIWI2, which is loaded with a piRNA, presumably via complementarity of the piRNA to their nascent transcripts. A set of proteins are key to stabilising MIWI2 and initiating silencing (e.g. SPOCD1), however, DNMT3C's interactor LSD1 is responsible for removing inhibitory H3K4me3 and allowing the *de novo* methylation that ensures stable epigenetic silencing.

drive the activity of a TE as a selfish promoter or an enhancer of gene expression. We are now investigating how these factors are important for genome regulation during development and how TEs exploit them for their selfish activity.

Novel regulators of the transposon 'life cycle' in pluripotent and differentiated stages of mammalian development

The possibility of using CRISPR-Cas9 to conduct forward genetic screens has opened up the opportunity to identify novel factors that regulate TEs and their life cycle in mammalian cells. However, as in any genetic screen, the devil is in the details: the phenotypic changes induced by the mutations might be difficult to use as a readout to select mutants. We came up with a strategy to select mutants that accumulate endogenous mRNAs from TEs and translate these mRNAs into TE proteins. We have now produced cell lines in which TE activity can be manipulated to fine-tune the readout of their molecular phenotypes prior to genetic screening.

FUTURE DIRECTIONS

The achievements outlined above will allow us to dive deeper into mechanistic studies focused on understanding how epigenetic settings are laid out at TE promoters in mouse germ cells and how this impacts the behaviour of germ cells during gametogenesis. The lab has developed and optimised tools for applying epigenomics to very specific cell types across gametogenesis. We will develop mouse models to couple these genome-wide studies with functional genomic studies.
We are also excited to continue exploring the relationship between TEs and DNA damage and repair using the cell lines in which TE activity can be controlled and hope to present exciting novel data about this relationship in stem cells in the upcoming year.

SELECTED PUBLICATIONS

Prakash SA and Barau J (2021) Chromatin profiling in mouse embryonic germ cells by CUT&RUN. Pages 253–264 in: Epigenetic reprogramming during mouse embryogenesis. Methods in Molecular Biology, vol 2214 (eds. Ancelin K & Borensztein M), *Springer US*, New York

Barau J, Teissandier A, Zamudio N, Roy S, Nalesso V, Hérault Y, Guillou F and Bourc'his D (2016) The DNA methyltransferase DNMT3C protects male germ cells from transposon activity. *Science*, 354:909–912

Zamudio N, Barau J, Teissandier A, Walter M, Borsos M, Servant N and Bourc'his D (2015) DNA methylation restrains transposons from adopting a chromatin signature permissive for meiotic recombination. *Genes Dev*, 29:1256–1270

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PETER **BAUMANN**

OVERVIEW

Elucidating the mechanisms by which chromosome ends are protected and maintained is a matter of great interest with far-reaching implications for a broad spectrum of age-related diseases including cancer, cardiovascular disease and immune senescence. Our telomere-related work centres around two key topics: (i) the mechanistic basis of chromosome end protection, and (ii) the biogenesis and regulation of telomerase. Our research is guided by the conviction that a better understanding of telomerase biogenesis and its regulation will lead to the identification of compounds that modulate telomere length. Such reagents will have therapeutic uses either to limit the proliferation of tumour cells or to boost the proliferative potential of cell populations needed for tissue renewal. The latter could not only help patients with telomeropathies, but may also counteract many of the detrimental phenotypes associated with normal ageing, such as increased susceptibility to infections and diminished response to vaccinations. To reach these goals, we employ genetic, molecular and cell biological approaches, and have built a network of collaboration partners to examine telomere dynamics in the physiological contexts of immune senescence, frailty and ageing of the enteric nervous system.

The amount of telomerase in a cell directly impacts its renewal capacity and ageing

RESEARCH HIGHLIGHTS

Telomerase biogenesis and regulation

Progressive telomere shortening is intrinsically linked to cell division and critically short telomeres trigger cellular senescence to prevent further proliferation. Mechanisms that replenish telomeric sequences are a double-edged sword. They extend the replicative lifespan of a cell population and are vital for tissue homeostasis. On the other hand, replenishing telomeres also permits the continued proliferation of malignant cells. Consequently, telomere addition must be tightly regulated. The isolation of the telomerase RNA subunit (TER1) from fission yeast by our laboratory provided a key tool for studying the biogenesis and regulation of the enzyme in a genetically tractable organism. This has led to a series of discoveries in telomere and RNA biology and has established a paradigm for telomerase assembly. Continuing our characterisation of the stepwise assembly process of telomerase, we showed that Pof8 is a La-related protein and a constitutive component of telomerase. We recently identified

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Early Career Scientist, Howard Hughes Medical Institute, Kansas City

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Associate Investigator, Stowers Institute for Medical Research, Kansas City

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Assistant Investigator, Stowers Institute for Medical Research, Kansas City

1998 – 2002
Research Associate, University of Colorado, Boulder

EDUCATION

1998
PhD in Biochemistry, University College London

1994
MPhil, University of Cambridge

two additional factors that form a complex with Pof8 and participate in telomerase assembly. One shares structural similarity with the nuclear cap-binding complex, the other is the orthologue of the methyl phosphate capping enzyme (Bin3/MePCE). Based on functional characterisation and structural similarities, we named the factors Thc1 (Telomerase Holoenzyme Component 1) and Bmc1 (Bin3/MePCE 1), respectively. Thc1 and Bmc1 function together with Pof8 in recognising correctly folded telomerase RNA and promoting the recruitment of the Lsm2-8 complex and the catalytic subunit to assemble functional telomerase (Figure 1).

While our studies on telomerase and telomeres in fission yeast have provided fundamental insights into chromosome end maintenance, we ultimately view this work as a stepping-stone towards understanding telomere maintenance in human cells. Recent work in our group uncovered roles for several RNA processing factors and an unusual RNA structure called a triple helix in the biogenesis of human telomerase. Importantly, our studies showed that maturation of human telomerase RNA (hTR) is in kinetic competition with its degradation, an observation that hints at potential treatment options for telomerase insufficiency disorders and for delaying or reversing certain degenerative processes associated with ageing.

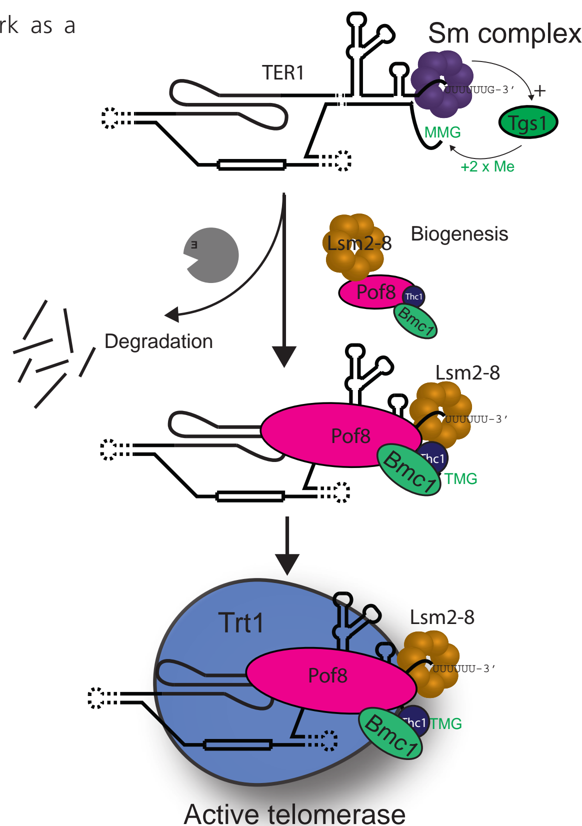

Figure 1. Telomerase biogenesis follows a hierarchical assembly process that stands in kinetic competition with degradation of the intermediates. After spliceosomal cleavage, the Sm complex (purple) facilitates cap hypermethylation by Tgs1 and is subsequently replaced by the Lsm2-8 complex (brown). Pof8, Thc1 and Bmc1 function during the Sm to Lsm transition. Pof8 (pink) promotes the loading of Lsm2-8 onto TER1, while Bmc1 (green) and Thc1 (blue) stabilise Pof8 binding through protein-protein and protein-RNA interactions. Only correctly folded and assembled intermediates are subsequently bound by the catalytic subunit of telomerase.

FUTURE DIRECTIONS

To gain a comprehensive understanding of human telomerase biogenesis, regulation and turnover, present studies are aimed at identifying additional factors and using biochemical and genetic means to elucidate their functions. Unravelling how telomerase is made and regulated has led us to several exciting questions: Can we modulate telomerase activity by manipulating RNA processing events? Is increasing the levels of telomerase a genuine path towards the treatment of premature ageing diseases? Does increased telomerase activity contribute to resilience and delay the onset of degenerative processes associated with normal ageing?

GROUP MEMBERS

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Technician; since 03/2021

SELECTED PUBLICATIONS

Páez-Moscoso DJ, Ho DV, Pan L, Hildebrand K, Jensen KL, Levy MJ, Florens L and Baumann P (2021) A putative cap binding protein and the methyl phosphate capping enzyme Bin3/MePCE function in telomerase biogenesis. *Nat Commun*, in press

Páez-Moscoso DJ, Pan L, Sigauke RF, Schroeder MR, Tang W and Baumann P (2018) Pof8 is a La-related protein and a constitutive component of telomerase in fission yeast. *Nat Commun*, 9:587

Tseng CK, Wang HF, Schroeder MR and Baumann P (2018) The H/ACA complex disrupts triplex in hTR precursor to permit processing by RRP6 and PARN. *Nat Commun*, 9:5430

PETRA **BELI**

OVERVIEW

Genome maintenance is essential for gene expression fidelity, as well as the prevention of cancer and premature ageing. The research in our group focuses on identifying and characterising proteins and signalling pathways that counteract genomic instability. A complex network of proteins and signalling pathways ensures genome maintenance in response to external stressors and DNA lesions introduced through cellular metabolism, as well as DNA replication and transcription. We develop and employ quantitative mass spectrometry-based approaches to obtain a systematic view of the proteins and signalling pathways that counteract genomic instability arising from cell-intrinsic sources, as well as in response to external stressors such as ultraviolet irradiation.

We investigate the mechanisms that maintain genome stability

RESEARCH HIGHLIGHTS

Transcription by RNA polymerase II is essential for all cellular processes and hence for the adaptive response of cells to internal and external stimuli. Dysregulated transcription resulting in high transcription rates and increased frequency of transcription-replication conflicts is observed in many tumours. R-loops are three-stranded nucleic acid structures formed by an RNA-DNA hybrid with a displaced non-template DNA strand. In addition to the regulatory functions of R-loops in transcription, DNA repair, telomere maintenance and chromosome segregation, these non-B DNA structures can be a driver of genomic instability. In cycling cells, R-loops are an obstacle to the replication machinery and can potentially result in transcription-replication conflicts that lead to fork breakage and DNA double-strand breaks. Furthermore, stalled transcription complexes at R-loops can trigger processing by nucleotide-excision repair endonucleases to form DNA double-strand breaks. Tight regulation of R-loop levels across the genome is essential for their function in promoting chromatin-associated processes and for preventing R-loop-dependent genomic instability.

To gain insights into the protein-based mechanisms that regulate R-loop homeostasis, we probed R-loop-proximal protein networks in a native chromatin environment using RNA-DNA proximity proteomics (RDProx). We fused the hybrid-binding domain of RNaseH1 to an engineered variant of soybean ascorbate peroxidase (APEX2) and identified previously known R-loop regulators such as TOP1, AQR, the single-stranded DNA-binding proteins RPA1/2 and

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Since 2020
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2013 – 2020
Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz

2010 – 2013
Postdoctoral Fellow, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen

EDUCATION

2011
PhD in Biology, Goethe University Frankfurt

2007
MSc in Molecular Biology, University of Zagreb

RNaseH2A, as well as components of the THO complex and the nuclear exosome. These R-loop proximal proteins showed functional interactions, as demonstrated by the identification of different protein clusters involved in splicing, m6A regulation, mRNA 3′ end processing, mRNA export, transcription regulation, chromatin organisation and DNA replication/repair. They were also enriched in domains typical for RNA- and DNA-binding proteins, including: RRM, helicase, DEAD/DEAH, CID, MCM N-terminal, RNA polymerase II-binding, SAP, MCM OB and K homology domains. Using RDProx, we identified a role for the tumour suppressor DEAD box protein 41 (DDX41) in opposing R-loop-dependent genomic instability. Depletion of DDX41 resulted in an accumulation of R-loops and DNA double-strand breaks at gene promoters as mapped by MapR and BLISS-sequencing, respectively. Germline loss-of-function mutations in DDX41 cause an increased predisposition to acute myeloid leukemia (AML) in adulthood. We propose that accumulation of R-loops at gene promoters, DNA double-strand breaks and the inflammatory response all contribute to the development of familial AML with mutated DDX41 (Mosler *et al*, *Nature Communications*, 2021).

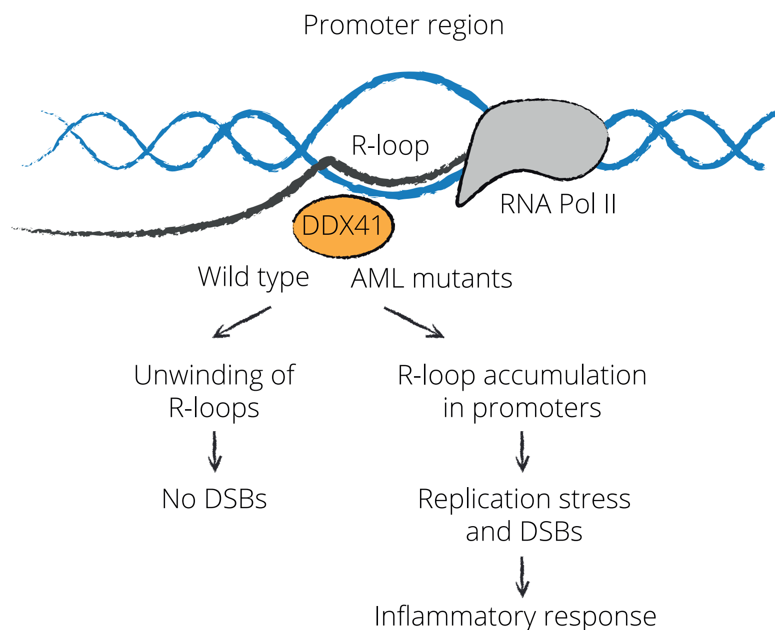

Figure 1. The function of the tumour suppressor Dead box helicase 41 (DDX41) in opposing transcription-associated R-loops and DNA double-strand breaks at promoters.

FUTURE DIRECTIONS

We will focus our future efforts on obtaining a proteomics view of the mechanisms that counteract genomic instability arising from cell-intrinsic sources. More than 10% of the human genome can fold into non-canonical (non-B) DNA structures, including R-loops and G-quadruplexes that can regulate different cellular processes. Non-canonical DNA structures also obstruct DNA replication and transcription, thereby creating an intrinsic source of DNA double-strand breaks and genomic instability. Another direction will be to further develop and employ multiplexed phosphoproteomics approaches to delineate the interplay between genomic instability and immune signalling pathways.

GROUP MEMBERS

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SELECTED PUBLICATIONS

Hildebrandt A, Brüggemann M, Rücklé C, Boerner S, Heidelberger JB, Busch A, Hänel H, Voigt A, Möckel MM, Ebersberger S, Scholz A, Dold A, Schmid T, Ebersberger I, Roignant JY, Zarnack K#, König J# and Beli P# (2019) The RNA-binding ubiquitin ligase MKRN1 functions in ribosome-associated quality control of poly(A) translation. *Genome Biol*, 20:216

Borisova ME, Voigt A, Tollenaere MAX, Sahu SK, Juretschke T, Kreim N, Mailand N, Choudhary C, Bekker-Jensen S, Akutsu M, Wagner SA and Beli P (2018) p38-MK2 signaling axis regulates RNA metabolism after UV-light-induced DNA damage. *Nat Commun*, 9:1017

Heidelberger JB, Voigt A, Borisova ME, Petrosino G, Ruf S, Wagner SA and Beli P (2018) Proteomic profiling of VCP substrates links VCP to K6-linked ubiquitylation and c-Myc function. *EMBO Rep*, 19:e44754

#indicates joint correspondence

FALK **BUTTER**

OVERVIEW

Mass spectrometry is a powerful tool for studying proteins in an unbiased and global manner. Current improvements in identification accuracy, sample throughput and data analysis allow the streamlined application of proteomics to answering diverse biological questions. Our group applies quantitative approaches such as label-free quantitation (LFQ), reductive demethylation (DML) and stable isotope labelling with amino acids in cell culture (SILAC), which enable us to directly compare thousands of proteins in complex mixtures. These technologies allow us to study changes in protein expression and are also applied in interactomics to identify specific protein interactions within a vast number of background binders. We apply mass spectrometry in several biological areas to advance our knowledge of cellular processes.

We discover new telomere-binding proteins using quantitative proteomics

RESEARCH HIGHLIGHTS

Phylointeractomics reveals evolutionary changes in protein binding

We developed a new experimental workflow for comparative evolutionary biology termed "phylointeractomics". In phylointeractomics, we interrogate a bait of interest with the proteomes of evolutionarily-related species in a systematic manner to uncover similarities and differences in protein binding. In a first application, we studied the telosome of 16 different vertebrate species ranging from zebrafish to humans, which spans a timeframe of 450 million years of evolution. While the telomeric sequence in vertebrates is a conserved TTAGGG repeat, there are some known variations of the interacting proteins, e.g. a *Pot1* gene duplication in the rodent lineage and an absence of *TIN2* in bird genomes. In our phylointeractomics screen, we recapitulated these evolutionary differences for the shelterin complex and additionally discovered that, contrary to predictions, not all homologues of TRF1 (a direct TTAGGG repeat-binding subunit of the complex) associated with our telomeric baits. Using recombinant TRF1 DNA-binding domains of even more vertebrate species, we located a gain-of-binding event at the branch point of the therian lineage, where mammals and marsupials diverged from monotremes such as the platypus. While TRF1 is present in most vertebrates, it seems to have obtained its telomeric function only later during vertebrate diversification. By exchanging selected amino acid residues in the platypus TRF1-DNA-binding domain, we could recreate a gain-of-binding switch *in vitro* that recapitulates a possible evolutionary scenario. Our

POSITIONS HELD

Since 2013
Group Leader, Institute of Molecular Biology (IMB), Mainz

2010 – 2013
Postdoc, Max Planck Institute for Biochemistry, Martinsried

EDUCATION

2010
PhD in Biochemistry, Ludwig Maximilian University (LMU), Munich

2006
Diploma in Biochemistry, University of Leipzig

phylointeractomics study therefore underscores that sequence homologues, as determined by phylogenomics, do not necessarily need to equate to functional homology.

Characterisation of new telomeric proteins

We use quantitative interactomics to identify new telomeric proteins. In addition to HOT1, we reported that the zinc finger protein ZBTB48 is a telomeric protein in mammals. Recently, we characterised ZBTB10 as a telomeric protein that preferentially binds to telomeres elongated by the alternative lengthening pathway. The extension of our workflow to other model species resulted in the identification of more novel telomere binding proteins. For example, we have just identified the long-sought double-strand telomere binding proteins TEBP-1 and TEBP-2 in the nematode *C. elegans*. We showed that both proteins modulate telomere length, but TEBP-1 CRISPR knockout strains have longer telomeres while TEBP-2 knockout strains have shorter telomeres and exhibit a mortal germline phenotype. Notably, both double-strand telomere-binding proteins form a complex with the known *C. elegans* single-strand binder POT-1, defining the first known telomere complex in nematodes.

Systems approaches to study developmental gene regulation

To study proteome dynamics during development, we generated two large developmental proteomic datasets for *Drosophila melanogaster*: a full life cycle dataset encompassing 15 different time points and a high temporal resolution proteome of embryogenesis. As both datasets match the previously published modENCODE developmental transcriptome, we systematically compared the developmental transcriptome and proteome and showed that in some cases, protein stability is the major determinant of protein levels. Additionally, we identified maternally loaded proteins, uncovered peptides originating from small open reading frames in lncRNAs, and resurrected the pseudogene Cyp9f3. The data is available to the research community via our web interface (www.butterlab.org/flydev).

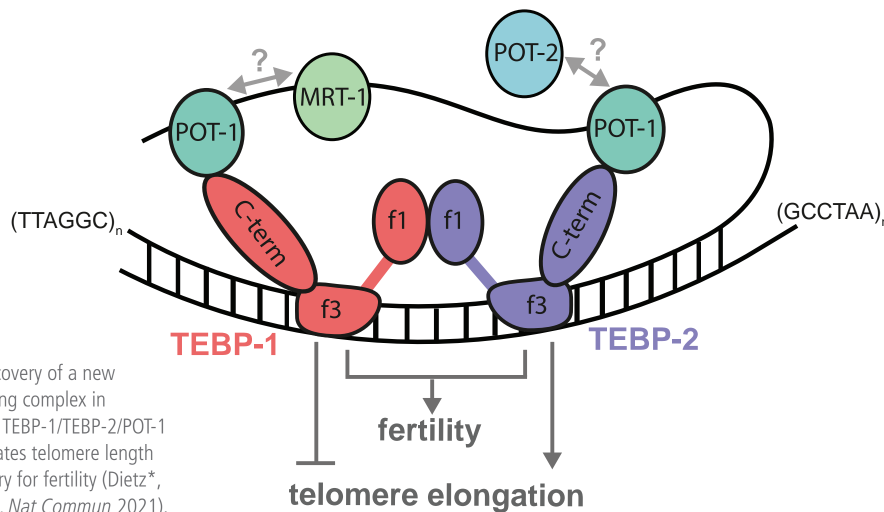

Figure 1. Discovery of a new telomere binding complex in *C. elegans*. The TEBP-1/TEBP-2/POT-1 complex regulates telomere length and is necessary for fertility (Dietz*, Almeida* *et al*, *Nat Commun* 2021).

FUTURE DIRECTIONS

We will continue to apply quantitative proteomics to diverse biological questions with a focus on differentiation, epigenetics, development and evolution. To this end, we are currently improving several parts of the proteomics and interactomics workflow established during the last few years in our group. By combining omics studies with classical biology, we are currently characterising novel telomeric proteins in diverse model species and investigating gene regulation in several eukaryotes.

GROUP MEMBERS

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Liudmyla Lototska
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Technician; since 02/2021

Emily Nischwitz
PhD Student; since 09/2018

Sarah Pawusch
PhD Student; since 10/2021

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SELECTED PUBLICATIONS

Dietz S*, Almeida MV*, Nischwitz E, Schreier J, Viceconte N, Fradera-Sola A, Renz C, Ceron-Noriega A, Ulrich HD, Kappei D, Ketting RF and Butter F (2021) The double-stranded DNA-binding proteins TEBP-1 and TEBP-2 form a telomeric complex with POT-1. *Nat Commun*, 12:2668

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*indicates joint contribution, #indicates joint correspondence

DOROTHEE **DORMANN**

OVERVIEW

Our research focuses on the molecular mechanisms of neurodegenerative diseases, most notably ALS (amyotrophic lateral sclerosis), FTD (frontotemporal dementia) and Alzheimer's disease. Existing therapies are designed to treat only the symptoms of disease, and no therapies are available to slow down or stop disease progression. The main objective of our research is to obtain a molecular understanding of the mechanisms underlying these devastating disorders. We seek to unravel how certain RNA-binding proteins (RBPs), in particular TDP-43 and FUS, become mislocalised and aggregated in these disorders, and how dysregulation of these RBPs causes a

We unravel the molecular mechanisms that cause neurodegenerative disorders

decline in cell function and neurodegeneration. Our previous research demonstrated that RBP mislocalisation and aggregation in ALS and FTD are intimately linked to molecular defects in 1) nuclear import, 2) control of liquid-liquid phase separation (LLPS) and 3) post-translational modifications (PTMs). We therefore study how nuclear transport, phase separation and PTMs of these disease-linked RBPs are normally regulated, how they are misregulated in disease and how these pathological changes could be reversed. By understanding the molecular mechanisms of protein mislocalisation and aggregation, we hope to help develop new therapeutic approaches to treat neurodegenerative diseases.

RESEARCH HIGHLIGHTS

The neurodegeneration-linked RBPs TDP-43 and FUS harbour extended intrinsically disordered regions (IDRs) that allow them to undergo weak multivalent self-self interactions, which can lead to LLPS and partitioning into cellular condensates, such as stress granules or other ribonucleoprotein (RNP) granules. Aberrant liquid-to-solid state transitions are believed to underlie the formation of pathological RBP aggregates in disease, however such aberrant phase transitions are normally efficiently suppressed by cellular quality control mechanisms. Our research has uncovered two important quality control mechanisms: regulation of RBP phase transitions by nuclear import receptors and PTMs.

Using *in vitro* reconstitution and cellular experiments, we showed that the nuclear import receptor of FUS (Transportin, TNPO1, also known as Karyopherin-ß2) efficiently suppresses LLPS and stress granule recruitment of FUS by interacting with the C-terminal nuclear localisation signal (NLS) and arginines in RGG/RG-rich regions, both of which are crucial drivers of FUS LLPS. We also found that in addition to Transportin, other importins (e.g. TNPO3 and

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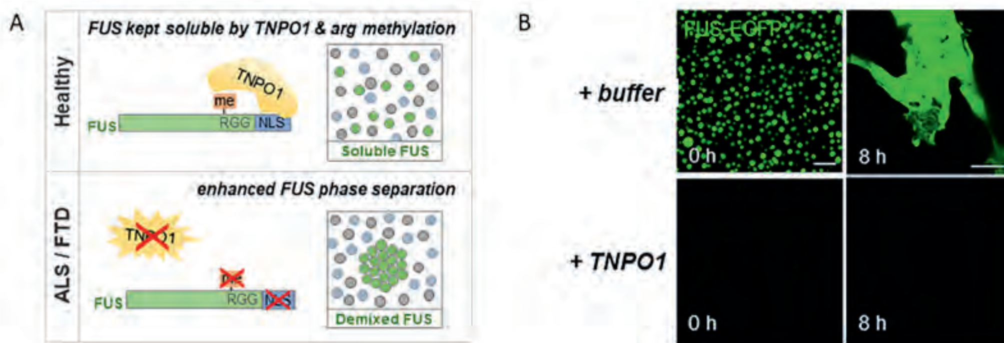

Figure 1. Regulation of FUS phase separation by its import receptor and arginine methylation.
(A) FUS phase separation is suppressed by the nuclear import receptor TNPO1 and post-translational arginine methylation. These control mechanisms are disrupted in the neurodegenerative diseases ALS and FTD.

(B) *In vitro* reconstitution experiment: Formation of FUS-EGFP droplets and aggregates *in vitro* in the absence or presence of equimolar amounts of purified TNPO1. Scale bar: 10 µm for 0h and 50 µm for 8h.

importin ß) bind directly to FUS via RGG/RG motifs and can suppress LLPS and stress granule recruitment of FUS, and that TNPO3 and importin ß can also import FUS into the nucleus, albeit with lower efficiency than Transportin. Based on these findings, we propose that FUS utilises a network of import receptors for chaperoning and import, similar to histones, ribosomal proteins and other RBPs we have investigated.

We also found that importins can directly bind and "shield" toxic arginine-rich dipeptide repeat proteins (poly-GR and -PR) that arise in the most common inherited form of ALS and FTD due to a hexanucleotide (GGGGCC) repeat expansion in the *C9orf72* gene. Poly-GR and -PR aberrantly interact with nucleic acids and promote LLPS of various RBPs, including TDP-43, however, these pathological interactions can be suppressed by elevated importin levels. Together, our work established an important role for importins in protein quality control as suppressors of aberrant phase transitions linked to disease. Thus, elevating importin levels or enhancing the binding of importins to aggregation-prone proteins could be a novel therapeutic strategy for protein aggregation disorders.

A second key regulator of RBP phase transitions that we have uncovered is disease-associated PTMs. PTMs frequently occur in IDRs and influence LLPS, but they can also affect RNA-binding properties and RNA-processing functions. Abnormal PTMs often arise in neurodegenerative diseases, e.g. TDP-43 is hyperphosphorylated and polyubiquitinated in ALS and FTD, and we previously found that arginine methylation of FUS RGG/RG motifs is reduced in FTD patients. Using *in vitro* methylation of purified FUS and phase separation assays, we found that FUS hypomethylation promotes LLPS and stress granule accumulation, suggesting that loss of this PTM may promote FUS aggregation in disease. More recently, we found that C-terminal TDP-43 hyperphosphorylation (which is known to be linked to disease) reduces TDP-43 phase separation and aggregation, renders TDP-43 condensates more dynamic and liquid-like, and suppresses its recruitment into cellular condensates (e.g. stress granules and nuclear bodies). This raises the possibility that TDP-43 phosphorylation may be a protective cellular mechanism for preventing TDP-43 aggregation and a physiological mechanism for regulating TDP-43 condensation.

FUTURE DIRECTIONS

We aim to further decipher at the molecular level how RBPs become dysfunctional in the context of neurodegenerative disorders and what consequences impaired importin function, aberrant LLPS and disease-linked PTMs have in cells. For instance, we plan to explore the cytosolic roles of importins more broadly by determining which proteins in the human proteome become insoluble in the cytoplasm upon acute importin depletion, and whether importins affect RNP granule dynamics or RNA binding/processing events in the cytoplasm. We also plan to identify and study potential new regulators of FUS and TDP-43 phase separation and aggregation (e.g. interacting proteins, nucleic acids, small molecules and PTMs), and to address whether the physiological functions of TDP-43 and FUS (e.g. in regulating splicing, R loops and DNA damage repair) require or are modulated by LLPS. By cooperating with polymer physicists and chemists, we seek to better understand the polymer and phase separation behaviour of various disease-linked proteins.

SELECTED PUBLICATIONS

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Hofweber M*, Hutten S*, Bourgeois B, Spreitzer E, Niedner-Boblenz A, Schifferer M, Ruepp MD, Simons M, Niessing D, Madl T and Dormann D (2018) Phase separation of FUS is suppressed by its nuclear import receptor and arginine methylation. *Cell*, 173:706–719.e13

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CLAUDIA **KELLER VALSECCHI**

OVERVIEW

A characteristic feature of diploid organisms is that the chromosomes are present in proportional amounts with respect to one other. Gene dosage alterations can be reflected in the amount of their expression products and thereby impact the cellular processes in which they play a role. For example, a principal factor of miscarriage in humans is aneuploidy, a condition where chromosomes occur in different numbers than the normal two. Furthermore, exome sequencing studies reveal a growing number of rare developmental disorders caused by heterozygous mutations in individual genes. Hence, having exactly twofold gene dosage appears to be highly relevant for proper cellular and organismal function.

We investigate gene dosage effects in development & disease

Paradoxically, gene dosage changes also occur naturally, for example in the form of polyploidy, which is a widespread phenomenon in plants and also occurs in fish, amphibians, reptiles and insects. Another example is the heteromorphic sex chromosomes, where degeneration of the Y/W can result in only a single functional allele being present in the heterogametic sex. Such dosage alterations can be buffered by cellular mechanisms such as dosage compensation. Using genome engineering, epigenomics, biochemistry and imaging, we investigate the causes and consequences of gene dosage alterations and their impact on developmental, pathogenic and evolutionary processes.

RESEARCH HIGHLIGHTS

Evolution of gene regulatory mechanisms of sex chromosomes

Heteromorphic sex chromosomes are a natural exception to the diploid state and can induce potentially deleterious gene expression imbalances. This is frequently corrected by a cellular mechanism termed dosage compensation (DC), which ensures equal expression of X-linked genes between males (XY) and females (XX). Sex chromosomes and their regulatory pathways show extremely high evolutionary turnover. We have recently compared DC in *Anopheles* and *Drosophila*, two closely related dipterans with similar gene content and whose X chromosomes evolved from the same ancestral autosome. Interestingly, we find that DC in *Anopheles* is achieved by an unknown molecular mechanism entirely different from that in *Drosophila*. To characterise this new mechanism, we created a sex-specific transcriptome atlas at various stages of mosquito embryogenesis. This revealed that DC is established progressively and completed towards the end of embryogenesis. It also allowed us to comprehensively describe the onset of sexually

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dimorphic gene expression. We have identified a candidate gene called *soa*, which may control DC. SOA is a nucleic acid-binding protein, and using immunofluorescence we found that it associates with a subnuclear territory akin to the X chromosome.

Gene paralogues and dosage-sensitivity of RNA-binding proteins
Dosage alterations occur not only at a chromosome-wide level, but also at the individual gene level. Gene duplication events are a key process in evolution because they create redundancy,

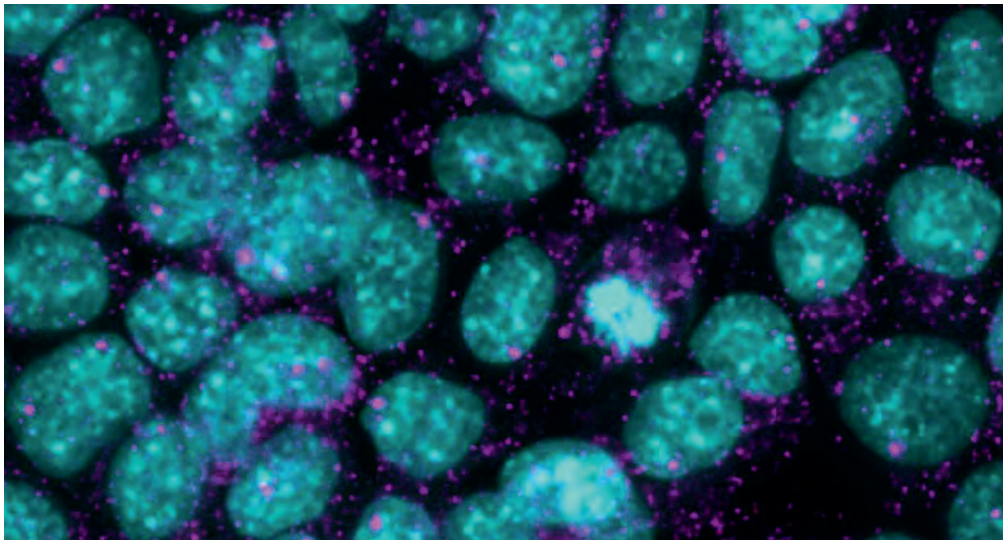

Figure 1. RNA FISH for an X-linked gene in male mouse embryonic stem cells.

In parallel with this work, we also aim to understand the complex biology of the mammalian sex chromosomes, where females inactivate one of their two X chromosomes. Despite X inactivation, some genes escape silencing and hence are expressed from both X chromosomes. This may provide an advantage to females with regards to an increase in gene dosage and higher allelic diversity. Because older individuals display an increased rate of mosaic chromosome losses, which most frequently affects the inactive X and the Y chromosomes, the loss of escapees may become relevant during ageing. We aim to develop a cellular system that will allow us to study this poorly characterised phenomenon in the context of ageing and its implications for cellular responses to environmental cues.

which liberates DNA sequences from previous selective constraints. One class of duplicated gene paralogues encodes dosage-sensitive RNA-binding proteins, components of the RNA spliceosome and splicing regulatory factors. Dosage alterations of the spliceosome components SNRPN and SNRPB can lead to disorders in humans that manifest with remarkably distinct phenotypes. We have created a heterologous system in which we characterised the dosage responses of these two paralogues. We found that exogenous expression of one paralogue triggers downregulation of the other paralogue and vice versa, indicative of crosstalk. This crosstalk appears to be dependent on an intrinsically disordered, low complexity region of the SNRPB/N proteins.

FUTURE DIRECTIONS

Our goal is to characterise DC mediated by SOA. Using genomics, we will identify how it regulates the X chromosome and create mutants to understand its relevance for DC. In addition to being only the 4th DC mechanism ever to be identified, these findings could ultimately inform novel strategies for fighting infectious diseases by vector control. We also plan to develop tools to comprehensively identify dosage-sensitive genes and cellular responses in mammals, for example those in SNRPB/N. For this, we will focus on the polymeric nature of RNA and low complexity regions. We will also expand our work on the mammalian X chromosome and study the mechanisms of re-activation during development, as well as age-related chromosomal mosaicism during ageing.

SELECTED PUBLICATIONS

Basilicata MF# and Keller Valsecchi CI# (2021) The good, the bad and the ugly: Evolutionary and pathological aspects of gene dosage alterations. *PLOS Genet*, 17:e1009906

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RENÉ **KETTING**

OVERVIEW

The major focus of my lab is gene regulation by small RNA molecules acting in RNAi-related pathways. Since their discovery at the start of the 21st century, many different RNAi-related pathways have been identified. It is now evident that although all of these pathways depend on proteins from the Argonaute family, each pathway has its own unique characteristics and effects on gene expression. These can range from relatively minor effects on translation (in the case of miRNAs) to full-blown shutdown of loci at the transcriptional level (piRNAs). We focus on mechanisms related to piRNA and siRNA biology, two species of small RNAs that are particularly abundant in, and important for, the germline. These small RNA pathways have a major role in maintaining genome integrity by controlling the activity of transposable elements. We use zebrafish and *C. elegans* as model systems to understand the molecular mechanisms governing these pathways and how they contribute to normal development. Questions such as how do small RNA pathways distinguish transposable elements from regular genes, how these pathways are organised at a sub-cellular level, and how small RNA populations can be inherited across generations are at the heart of our research.

We work to understand how non-coding RNAs resolve genetic conflicts

RESEARCH HIGHLIGHTS

First structural insights into piRNA biogenesis
The nematode *C. elegans* makes a special class of small RNAs that, when bound to a protein of the Piwi family, scan the genome for the presence of foreign sequences, triggering their silencing. Thus, these small RNAs act as specificity factors in a genomic immune system. These small RNAs, named 21U RNAs or piRNAs, are produced as tiny non-coding transcripts from tens of thousands of individual transcription units clustered in two large regions of the genome, resembling how other non-coding genes, e.g. tRNAs, rRNAs and snRNAs, are organised. To mature, the precursor piRNAs of *C. elegans* first need to be processed, losing nucleotides at both the 5′ and 3′ ends. While the enzyme responsible for processing the 3′ ends has been identified, nothing is known about 5′ end processing. In 2019, we identified a protein complex that binds to piRNA precursors and is essential for their maturation. We named this complex PETISCO – Portuguese for *tapas*. PETISCO consists of a number of subunits with different functions. During this year, in collaboration with the groups of Sebastian Falk (Max Perutz Laboratories, Vienna) and Janosch Hennig (EMBL, Heidelberg), we have been able to obtain the first structural insights into how PETISCO is organised and published the results

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2010 – 2013
Professor of Epigenetics in Development, University of Utrecht

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EDUCATION

2000
PhD in Molecular Biology, Netherlands Cancer Institute, Amsterdam

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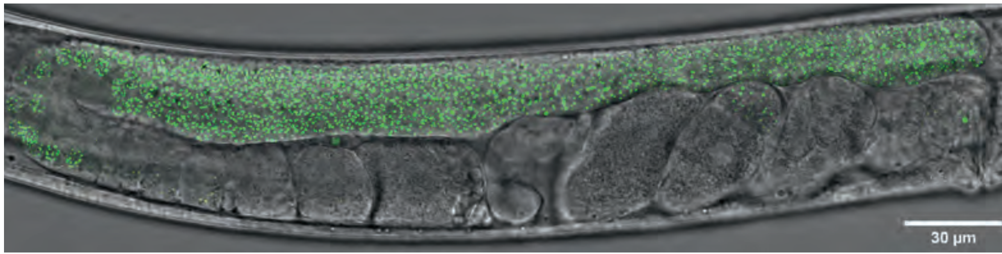

Figure 1. A gonad arm in *C. elegans* visualised by Nomarski microscopy and overlaid with fluorescent signal from the PGL-1 protein, which marks P granules (phase-separated entities). The gonad starts at the top-right, goes left and turns towards the bottom of the image. After this turn, the oocytes (which do not express strong PGL-1 signal) become visible. Following the spermatheca, four embryos are visible, just before the vulva (extreme bottom right).

in *Genes & Development*. We found that the complex forms a dimer of tetramers, and using both biochemistry and genetics, identified the important interfaces that hold this complex together. We also showed that the strongly conserved subunit ERH-2 (a homolog of enhancer of rudimentary) serves as an important dimerisation factor and as an interface for two different PETISCO effector proteins that steer PETISCO towards two different functions: one relates to piRNA biogenesis, while the other relates to embryonic development. Ongoing work on this intriguing complex will pursue the identification of its embryonic function and identification of the nuclease that processes piRNA precursors in PETISCO.

Massive non-coding RNA production during primordial germ cell development in zebrafish
We have also identified novel aspects related to germ cell development in zebrafish embryos. Germ cells have been sufficiently studied during the first day of development and after a number of weeks when they become mature germ cells, but the period in between remained uncharacterised. We found that in this time window the so-called primordial germ cells (PGCs) display unusual nuclear morphologies and overall open chromatin. Using electron microscopy, we revealed that in this window in time the well-known perinuclear structures known as nuage change in morphology, and that this correlates with the onset of zygotic piRNA production. Using sequencing approaches, we also showed that during this phase of PGC development, large genomic loci called PERLs are transcribed and that PERL transcripts are found very close to known sites of piRNA production: nuage. We proposed a model in which the maternally provided piRNA pool uses the PERL transcripts to initiate zygotic piRNA production. These results were published in *Development*.

FUTURE DIRECTIONS

Future work will continue to mechanistically unravel the molecular pathways which are steered by small RNA molecules. One current emphasis is on the inheritance of small RNA-driven responses, and we have recently uncovered a novel mechanism by which this can happen via the male sperm. This mechanism is driven by a process known as phase separation, and a future aim will be to understand how this mechanism can release the silencing information in the fertilised oocyte. We will also study additional piRNA-related condensates and how they are regulated during germ cell development and embryogenesis in *C. elegans* and zebrafish. We will increasingly use biochemistry to look at phase separation-related processes, as well as the processing of small RNAs. Finally, we will continue to use genetic screens and are currently planning one to better understand the embryonic function of PETISCO, as our results indicate that this function could be strongly conserved and of broad relevance.

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SELECTED PUBLICATIONS

Cordeiro Rodrigues RJ, de Jesus Domingues AM, Hellmann S, Dietz S, de Albuquerque BFM, Renz C, Ulrich HD, Sarkies P, Butter F and Ketting RF (2019) PETISCO is a novel protein complex required for 21U RNA biogenesis and embryonic viability. *Genes Dev*, 33:857–870

Almeida MV, Dietz S, Redl S, Karaulanov E, Hildebrandt A, Renz C, Ulrich HD, König J, Butter F and Ketting RF (2018) GTSF-1 is required for formation of a functional RNA-dependent RNA polymerase complex in *Caenorhabditis elegans*. *EMBO J*, 37:e99325

Roovers EF*, Kaaij LJT*, Redl S, Bronkhorst AW, Wiebrands K, de Jesus Domingues AM, Huang H, Han C, Riemer S, Dosch R, Salvenmoser W, Grün D, Butter F, van Oudenaarden A, Ketting RF (2018) Tdrd6a regulates the aggregation of Buc into functional subcellular compartments that drive germ cell specification. *Dev Cell* 46:285–301

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ANTON **KHMELINSKII**

OVERVIEW

Proteome integrity is maintained by a complex network that controls the synthesis, folding, transport and degradation of proteins. Numerous quality control systems that operate throughout the protein lifecycle prevent, detect and remove abnormal proteins, thus contributing to proteome homeostasis. Selective protein degradation by the ubiquitin-proteasome system (UPS) plays a key role in proteome turnover and quality control. When degradation is not possible, abnormal proteins can eventually be removed via asymmetric partitioning during cell division. Despite the activity of such systems, proteome homeostasis declines with ageing and in numerous diseases, resulting in the accumulation of abnormal proteins and loss of cell functionality.

We use proteomics and fluorescent timers to determine how cells recycle proteins

Working in yeast and human cells, we aim to systematically examine how cells deal with different types of abnormal proteins. We use genetic and proteomic approaches that exploit fluorescent timers to identify substrates of the various UPS components and to explore the functions of this system in replicative ageing and genome stability. Our goals are to understand the coordination between protein biogenesis and quality control, to decipher how abnormal proteins are recognised, and to elucidate how cells adapt to challenges in proteome homeostasis.

RESEARCH HIGHLIGHTS

Selective protein degradation is involved in most cellular processes and contributes to proteome homeostasis through the removal of unnecessary or abnormal proteins. The UPS is the key system of selective protein degradation, whereby a cascade of ubiquitin-activating (E1), ubiquitin-conjugating (E2) and ubiquitin-protein ligase (E3) enzymes marks proteins with polyubiquitin chains for degradation by the proteasome. Deubiquitinating enzymes (DUBs), which remove ubiquitin marks and replenish the pool of free ubiquitin, are involved at various stages of the targeting and degradation processes. Despite the central role of the UPS in protein degradation and its association with various diseases and ageing, the functions of many UPS components are unclear and the substrate specificities of E3s and DUBs are not well defined.

To address these limitations, we sought to systematically assess the role of UPS components in proteome turnover using budding yeast as a model. We established a proteomic approach based on fluorescent timers and examined how inactivation of individual UPS

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EDUCATION

2010
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components affects proteome abundance and turnover (Figure 1a). We have now analysed the state of the yeast proteome in mutants of almost all known E2, E3 and DUB enzymes and have observed phenotypes for 76% of them (Kong *et al*, 2021). This effort yielded a rich dataset to explore the different roles of the UPS from the perspective of a protein of interest or with a UPS component as a starting point.

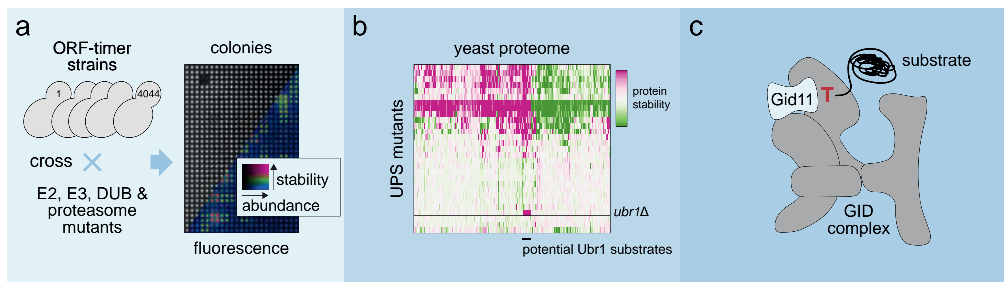

Figure 1. Systematic characterisation of the ubiquitin-proteasome system. **a)** Proteomic profiling of the yeast UPS in colony arrays expressing different fluorescent timer-tagged proteins and carrying mutations in individual UPS components. **b)** Identification of UPS substrates based on changes in protein stability. **c)** Model of Gid11 as a GID receptor for substrates with an N-terminal threonine.

We used this dataset of potential UPS substrates and functions to gain insights into N-degron pathways, which target proteins carrying N-terminal degradation signals (N-degrons) for proteasomal degradation. On the one hand, we found that the conserved cytosolic E3 Ubr1 has a role in the degradation of mitochondrial proteins processed by the mitochondrial inner membrane peptidase Imp1 (Figure 1b). Imp1 cleaves N-terminal signal peptides from proteins targeted to the mitochondrial intermembrane space. Our results suggest that incompletely imported intermembrane space proteins can still be processed by Imp1 but are subsequently released back into the cytosol, exposing an N-degron at the new N-terminus, which is then recognised by Ubr1. On the other hand, we discovered a new substrate receptor for the GID ubiquitin ligase (Figure 1c). The GID/CTLH complex is a large multisubunit E3 that appears to use interchangeable receptor subunits for substrate recognition via N-degrons. In yeast, all previously identified GID substrates carried N-degrons specified by an N-terminal proline. Surprisingly, we identified 30 potential GID substrates that instead have an N-terminal threonine or serine. How are such substrates recognised by the GID E3? Genetic screens for turnover regulators of two such substrates, the nucleotidase Phm8 and the carbamoyl phosphate synthetase Cpa1, led us to the uncharacterised protein Ylr149c/Gid11. Using genetic and biochemical approaches, we found that Gid11 is a subunit of the GID complex that likely functions as a receptor to recognise substrates via threonine N-degrons (Figure 1c). Our work shows that Gid11 has a wide range of substrates, and based on this, we are eager to explore the potential roles of Gid11-dependent protein degradation in genome stability and metabolism.

FUTURE DIRECTIONS

We will continue our work on the GID complex to define its substrate repertoire, its different modes of substrate recognition and its physiological functions. In addition, we will expand our systematic characterisation of the UPS by combining our timer-based screens with mass spectrometry and adapt these approaches to human cells. We will apply these genetic and proteomic approaches to identify redundancies in the ubiquitin-proteasome system and, in this way, find substrates for overlapping degradation pathways. Finally, we will exploit our high-throughput approaches to understand how eukaryotic cells dispose of different types of abnormal proteins, including misfolded, mislocalised and orphan molecules.

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SELECTED PUBLICATIONS

Kong KYE*, Fischer B*, Meurer M*, Kats I, Li Z, Rühle F, Barry JD, Kirrmaier D, Chevyreva V, San Luis BJ, Costanzo M, Huber W, Andrews BJ, Boone C, Knop M# and Khmelinskii A# (2021) Timer-based proteomic profiling of the ubiquitin-proteasome system reveals a substrate receptor of the GID ubiquitin ligase. *Mol Cell*, 81:2460–2476

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*indicates joint contribution, #indicates joint correspondence

JULIAN KÖNIG

OVERVIEW

Posttranscriptional gene regulation plays an important role not only in development and tissue identity but also in neurodegenerative diseases and cancer. The fate of mRNA is regulated by the cooperative action of RNA-binding proteins (RBPs), which recognise specific RNA sequences to form messenger ribonucleoprotein complexes (mRNPs). The information in the RNA sequence and how it is interpreted by RBPs is commonly referred to as the 'mRNP code'. However, the molecular features that define this code remain poorly understood.

My main goal is to significantly contribute to cracking the mRNP code. To this end, we focus on the molecular mechanisms of splicing regulation and ribosome-associated quality control of translation and their contribution to human physiology and disease.

We dissect splicing networks using in vitro iCLIP technology

RESEARCH HIGHLIGHTS

Development of *in vitro* iCLIP for high-throughput measurements of protein-RNA binding

Understanding mRNPs and mRNP-mediated regulation requires profound knowledge of the interplay of *cis*-regulatory elements, *trans*-acting RBPs and large cellular machineries like the spliceosome and ribosome. To build this knowledge, my group has developed a new technology: in order to study the intrinsic RNA-binding activity of RBPs, we established *'in vitro* iCLIP' experiments, in which recombinant RBPs are incubated with long transcripts (Sutandy *et al*, 2018). Using this technology, we addressed the RNA binding of the essential splicing factor U2AF2, which recognises the 3' splice sites of exons. We measured U2AF2 affinities at hundreds of binding sites and compared *in vitro* and *in vivo* binding landscapes using mathematical modelling. We found that *trans*-acting RBPs extensively regulate U2AF2 binding *in vivo*, including enhanced recruitment to 3' splice sites and clearance of introns (Figure 1). Using machine learning, we identified and experimentally validated novel *trans*-acting RBPs such as FUBP1, BRUNOL6 and PCBP1, which modulate U2AF2 binding and affect splicing outcomes. Our study offers a blueprint for the high-throughput characterisation of *in vitro* mRNP assembly and *in vivo* splicing regulation.

POSITIONS HELD

Since 2013
Group Leader, Institute of Molecular Biology (IMB), Mainz

2008 – 2013
Postdoc, MRC Laboratory of Molecular Biology, Cambridge

EDUCATION

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PhD in Biology, Max Planck Institute for Terrestrial Microbiology & Philipps University, Marburg

2003
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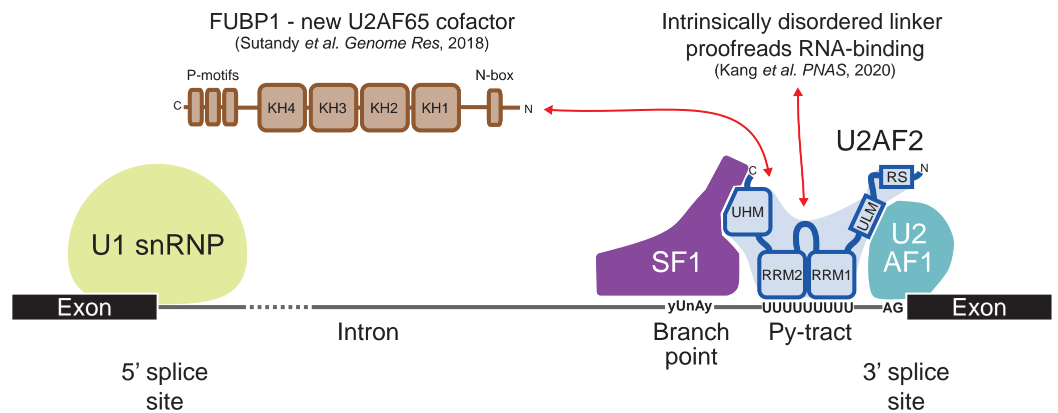

Figure 1. Early spliceosome assembly at the 5' and 3' splice sites.

An autoinhibitory intramolecular interaction proof-reads RNA recognition by the essential splicing factor U2AF2
In a recent study together with the Sattler group at the Technical University of Munich, we combined our *in vitro* iCLIP approach with NMR structural biology to identify a novel molecular mechanism for U2AF2 splicing regulation. The recognition of splice sites is initiated by stable U2AF2 binding to the poly-pyrimidine tract (Py-tract) upstream of exons to assemble the spliceosome. However, it remains unclear how U2AF2 discriminates between weak and strong Py-tract RNAs. We found that the intrinsically disordered linker region connecting the two RNA recognition motif (RRM) domains of U2AF2 mediates autoinhibitory intramolecular interactions that reduce non-productive binding to weak Py-tract RNAs. This proofreading favours the binding of U2AF2 at stronger Py-tracts, which is required to define 3' splice sites at the early stages of spliceosome assembly. Mutations that impair the linker autoinhibition enhance U2AF2 affinity for weak Py-tracts, resulting in promiscuous binding along mRNAs and impacting splicing fidelity. Our findings highlight an important role for intrinsically disordered linkers in modulating RNA interactions of multi-domain RBPs.

FUTURE DIRECTIONS

My research will focus on deciphering the regulatory code of splicing and quality control mechanisms in human physiology and disease. To this end, we will build on the iCLIP technology to map protein-RNA interaction sites throughout the transcriptome. We will use our approaches to predict mutations that cause mis-splicing in cancer and neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). We will also take a closer look at critical RNA regulators that are relevant in neurodegeneration. For instance, we recently showed that small alterations in the cellular concentration of the RNA-binding protein HNRNPH can have a strong impact on alternative splicing events in diseases caused by nuclear aggregation. Following up on the underlying molecular mechanisms, we will analyse the contribution of multivalent interactions and phase separation to this switch-like regulation. Beyond splicing, we will investigate the functional connections between RNA-binding proteins that exhibit E3 ubiquitin ligase activity and their role in ribosome-associated quality control of poly(A) translation.

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NARD **KUBBEN**

OVERVIEW

Ageing is a prime pathological component of most prevalent diseases. At the cellular level, it is characterised by various hallmarks, including epigenetic alterations, genomic instability and loss of protein homeostasis, all of which contribute to an organism-wide decline in function. Unfortunately, our current knowledge of the molecular pathways that drive cellular ageing and the formation of ageing hallmarks is severely limited. Our lab's work focuses on uncovering the fundamental biological mechanisms of ageing that can be manipulated to slow down the progression of ageing-related diseases, including the rare, lethal premature ageing disease Hutchinson-Gilford

We use high-throughput screening to identify new anti-ageing mechanisms

Progeria Syndrome (HGPS). Our group is employing unbiased genomics, proteomics and high-throughput microscopy-based screening approaches to: 1) identify novel pathways that slow down the onset of cellular ageing; 2) investigate cellular pathways that help reverse ageing defects that have already formed, and 3) validate the therapeutic potential of identified ageing mechanisms across various model systems of ageing-related diseases. The overarching goal of our research is to uncover fundamental biological mechanisms of ageing that can help improve human healthspan.

RESEARCH HIGHLIGHTS

A novel model system to identify drivers of ageing

One of the major challenges of ageing research is that ageing manifests as a slow build-up of relatively low percentages of aged cells in our bodies. Molecular techniques that aim to identify causes of ageing by directly comparing young and aged biological tissue samples therefore have the disadvantage of only detecting the most robust ageing-correlated changes, many of which turn out to be a consequence rather than a cause of ageing. It is therefore key to establish a technical approach that excludes these passive bystander effects of ageing and focuses directly on identifying mechanisms that actively drive ageing.

We have therefore established an HGPS-based system to functionally screen for events that drive ageing. HGPS is predominantly caused by a silent mutation in the *LMNA* gene, which encodes the nuclear lamina-localised protein lamin A, a key organiser of the mammalian nucleus. The silent mutation in HGPS results in the accumulation of an alternatively spliced lamin A mutant, termed progerin. A more modest accumulation of progerin also occurs during physiological ageing, which

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EDUCATION

2010
PhD in Molecular Biology, Maastricht University, The Netherlands

2004
MSc in Biological Health Sciences, Maastricht University, The Netherlands

2001
BS in Health Sciences, Maastricht University, The Netherlands

suggests that HGPS and physiological ageing have a common mechanistic basis. Unfortunately, the mechanisms by which progerin exerts its dominant negative effects remain largely unknown. We have now generated a cellular system in which we can inducibly express progerin and study the formation of many cellular ageing defects within a convenient time-frame of only 4 days, using a semi-automated high-throughput microscopy pipeline to visualise and quantify ageing defects. This system enables us to investigate whether any type of drug or gene-targeting intervention can prevent ageing upon progerin expression, thereby identifying pathways that are directly involved in driving the cellular ageing process.

High-throughput identification of new anti-ageing targets

Using our HGPS-based system, we screened a library of 2,816 FDA-approved compounds for their ability to prevent the formation of ageing defects. We found that retinoids depotentiate progerin from causing cellular ageing by inhibiting the expression of the *LMNA* gene. We furthermore screened a siRNA library targeting 320 human ubiquitin ligases for their capacity to prevent the formation of ageing defects and identified a novel involvement of the NRF2 pathway in progerin-induced ageing. The transcription factor NRF2 is a master regulator of various pathways that promote cellular homeostasis, including the antioxidative defence system, which protects cells against oxidative stress. We found that progerin erroneously aggregates in the nucleus, entrapping NRF2 proteins and thereby preventing transcriptional activation of NRF2-regulated antioxidative genes. Reactivation of the NRF2 pathway's activity through the FDA-approved compound Oltipraz significantly slowed down the formation of ageing defects upon progerin expression and even helped partially reverse ageing defects that already had been established in both cellular and *in vivo* mouse models. Furthermore, direct impairment of the NRF2 pathway was sufficient to drive the formation of various cellular ageing hallmarks. Our work has therefore provided proof-of-principle that HGPS-based high-throughput screening approaches can be used to identify novel ageing mechanisms that may have therapeutic uses in slowing down ageing.

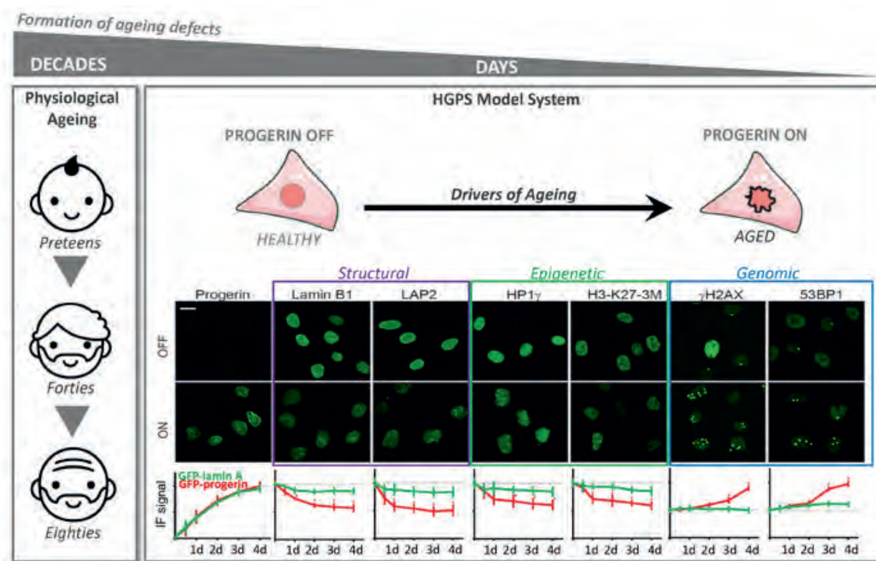

Figure 1. An overview of the inducible cellular HGPS model in comparison to other forms of ageing. The manifestation of ageing defects on average takes decades during physiological ageing, years in the premature ageing disease HGPS, and 4 days in the HGPS cellular system. Structural, epigenetic and genomic defects are induced by progerin, but not by wild-type lamin A expression.

FUTURE DIRECTIONS

Our future work will continue to mechanistically unravel the molecular pathways that regulate ageing. We are currently performing a genome-wide RNAi screen to identify novel factors that prevent the formation of proteostatic and DNA damage ageing defects upon expression of progerin. We anticipate that this screen will identify approximately 50-100 new drivers of ageing. We will further investigate these candidates for their ability to prevent and reverse the formation of a wide variety of ageing defects in both HGPS and physiologically aged cells. Complementary to this screen, which investigates the anti-ageing effects of reduced expression of target genes, we plan on establishing a new CRISPR-Cas9 based model system that will determine which genes have anti-ageing effects upon increased expression. We will further deepen our mechanistic understanding of how progerin affects cellular proteostasis through a variety of genomics and proteomics- driven studies. Lastly, we plan on investigating the role of the newly identified ageing mechanisms in animal models of ageing.

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PhD Student; since 05/2021

Verena Wilhelm
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EDWARD **LEMKE**

OVERVIEW

We focus on studying intrinsically disordered proteins (IDPs), which constitute up to 50% of the eukaryotic proteome. Paradoxically, they are most famous for their involvement in misfolding in neurodegenerative diseases of ageing like Alzheimer's, Parkinson's and Huntington's disease. However, IDPs are key in many vital biological processes, such as nucleocytoplasmic transport, transcription and gene regulation. The ability of IDPs to exist in multiple conformations is considered a major driving force behind their enrichment during evolution in eukaryotes. Studying biological machineries containing such dynamic proteins is a major hurdle for conventional technologies. Because of this, and due to the fact that they are hard to visualise, IDPs are termed the dark proteome. Using a question-driven, multidisciplinary approach paired with novel tool development, we have made major strides in understanding the biological dynamics of such systems from the single molecule to the whole cell level. Fluorescence tools are ideally suited to study the plasticity of IDPs, since their non-invasive character permits smooth transition between *in vitro* (biochemical) and *in vivo* (in cell) studies. In particular, single molecule and super-resolution techniques are powerful tools for studying the spatial and temporal heterogeneities that are intrinsic to complex biological systems. We synergistically combine this effort with cutting-edge developments in chemical and synthetic biology, microfluidics and microscope engineering to increase the throughput, strength and sensitivity of the approach as a whole.

We study how disorder allows proteins to perform multiple functions with precision

RESEARCH HIGHLIGHTS

Our strong focus on understanding the mechanisms of IDP function is both driven by and driving novel tool developments for "in-cell/*in situ* structural biology." This comprises a synergistic effort of chemical/synthetic biology and precision fluorescence-based technology/nanoscopy/single-molecule/super-resolution/microfluidics development.

A major technical breakthrough of my lab was the ability to engineer "click"able functionalities into any protein *in vitro* and *in vivo*. This genetic code expansion (GCE) approach has the potential to become a true GFP (fusion protein) surrogate strategy, with the major advantage being that superior synthetic dyes can be coupled with residue-specific precision anywhere in a protein. This opens up new avenues in single-molecule fluorescence and super-resolution

POSITIONS HELD

Since 2018

Adjunct Director, Institute of Molecular Biology (IMB), Mainz

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2009 – 2017

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EDUCATION

2005

PhD in Chemistry, Max Planck Institute for Biophysical Chemistry & University of Göttingen

2001

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2001

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microscopy. More recently, we have been able to merge our understanding of protein disorder and synthetic biology into the design of new membraneless organelles dedicated to protein engineering *in situ* (Figure 1). These custom organelles do not just execute a distinct second genetic code inside the cells; their bottom-up design also enables us to learn how phase separation can be used to generate new functions in eukaryotes. Our findings also have wider implications for understanding gene regulatory and stress-based mechanisms carried out by distinct, naturally-occurring organelles that play vital roles in regular cell function, as well as in ageing mechanisms.

These precision tools enable us to make even the most complex molecular machinery visible to our core methodologies, which are based on time-resolved multiparameter and nanoscopy tools. This allows innovative approaches to study the heterogeneity of IDPs. We discovered a distinct ultrafast protein-protein interaction mechanism that can explain how nuclear pore complexes (NPCs) efficiently fulfil their central role in cellular logistics, and how nuclear transport can be both fast and selective at the same time. We also used microfluidics to show how the permeability barrier of the nuclear envelope could be formed by liquid-liquid phase separation of a single disordered protein species. These findings provide a leap forward in our understanding of how IDPs can perform multiple functions through conformational changes, despite the normal requirement for rigid molecular specificity.

FUTURE DIRECTIONS

IDPs lack a stable structure and can easily misfold to the amyloid state and aggregate, resulting in their prominent role in many ageing-related diseases. This intrinsic risk must be outweighed by multiple advantages to explain their enrichment in the evolution of more complex species, but we are only at the beginning of understanding this. IDPs are highly multifunctional, and due to their multivalency and large, disordered regions they can function as dynamic scaffold platforms. We combine chemical and synthetic biology approaches to enable non-invasive, multi-colour high- and super-resolution studies of activity-dependent protein conformation changes in living cells, enabling fluorescence-driven *in situ* structural biology. The key point is that the enhanced spatial and temporal resolution offered by our fluorescent methods will enable us to detect rare events and unexpected behaviours inside cells, which we will then use to identify and understand IDP multifunctionalities that are clearly distinguishable from their normal mode of action. For example, nucleoporins (Nups) normally function in the nuclear pore complex (NPC), but in fact many IDP-Nups have diverse roles, such as in pathogen-host interactions, and can even shuttle away from the NPC to function in gene regulatory processes. Fusions of Nup98 with transcription factors, for example, are known to be linked to leukaemia.

Our work is accompanied by rigorous analysis of the physicochemical properties of IDPs and examines to what extent simple, known polymer concepts such as phase separation can be used to describe the function of IDP biopolymers *in vivo*. Vice versa, we are particularly interested in how disordered proteins play key roles in gene regulation and cellular ageing.

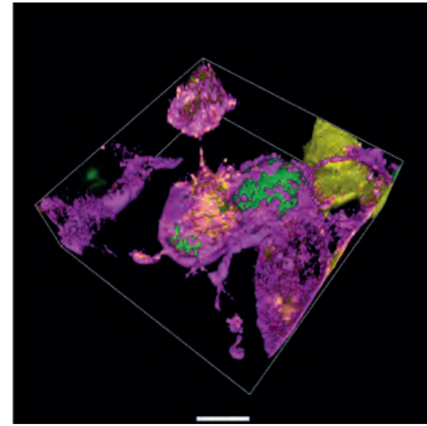

Figure 1. Membraneless orthogonally translating (OT) organelles are designed organelles enriched in a suppressor tRNA/tRNA synthetase pair and a specific mRNA-binding domain (MCP) by means of protein condensation onto a membrane surface. This yields a thin film in which the genetic code has been changed to encode novel functionalities into proteins (nucleus in green, tRNA/tRNA synthetase in yellow and purple, in HEK293T cells. Adapted from Reinkemeier & Lemke, *Cell* 2021). Scale bar: 10 µm

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PhD Student; since 09/2019

Miao Yu
Postdoc; since 10/2018

SELECTED PUBLICATIONS

Reinkemeier CD and Lemke EA (2021) Dual film-like organelles enable spatial separation of orthogonal eukaryotic translation. *Cell*, 184:4886–4903.e21

Celetti G*, Paci G*, Caria J, VanDelinder V#, Bachand G#, Lemke EA# (2020) The liquid state of FG-nucleoporins mimics permeability barrier properties of nuclear pore complexes. *J Cell Biol* 219:e201907157

Reinkemeier CD*, Girona GE* and Lemke EA (2019) Designer membraneless organelles enable codon reassignment of selected mRNAs in eukaryotes. *Science*, 363:eaaw2644

*indicates joint contribution, #indicates joint correspondence

KATJA **LUCK**

OVERVIEW

Cells function because their molecular components, i.e. DNA, RNA, proteins, etc. interact with each other. This complex network of molecular interactions mediates all cellular functions and organisation. Genetic and environmental insults perturb these interactions and can cause disease. Because of technical limitations, we still lack a comprehensive and detailed structural and functional understanding of all the protein interactions in a human cell, hindering our ability to understand physiological and pathological molecular mechanisms. To tackle these limitations, my lab develops novel computational and experimental methods to identify the interfaces of protein interactions, and based on this, obtain information on the molecular function of the interaction. Furthermore, we explore the use of protein interaction interface information to predict the pathogenicity of genetic variants and develop integrative systems approaches to generate more specific mechanistic hypotheses that are suitable for experimental follow-up. We apply our approaches to proteins that are associated with neurodevelopmental disorders, focusing on implicated genome integrity processes and aiming to understand the physiological role of the proteins in these processes and the mechanisms that lead to disease when they are mutated.

We employ data-driven, systematic approaches to understand biological systems

RESEARCH HIGHLIGHTS

Prediction of protein interaction interfaces
Two common ways by which proteins can interact with each other are those where 1) the globular domains of two proteins bind to each other or 2) a globular domain (hereafter referred to as domain) in one protein binds to a short linear motif in a disordered region of another protein. Unfortunately, comprehensive and accurate information on the types of domain-domain and domain-motif interactions are not available, hindering their use in the prediction of these interface types in a given protein interaction. We have improved a manually curated list of domain-motif types and developed a predictor that uses this information to predict domain-motif interfaces in protein interactions. We have now started working on a complementary predictor for domain-domain interfaces. Additionally, we started using AlphaFold-2 to generate models of predicted interfaces and are exploring whether they can be used to increase the specificity of interface predictions and/or help in the design of mutations to experimentally validate predicted interfaces (see below).

POSITIONS HELD

Since 2020
Emmy Noether Group Leader, Institute of Molecular Biology (IMB), Mainz

2013 – 2019
Postdoctoral Fellow, Dana-Farber Cancer Institute and Harvard Medical School, USA

2007 – 2008
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EDUCATION

2012
PhD in Bioinformatics, University of Strasbourg

2007
Diploma in Bioinformatics, Friedrich Schiller University Jena

Validation of predicted protein interaction interfaces
Despite all efforts, predictions of protein interaction interfaces will not be 100% accurate and thus require experimental validation. We have set up a quantitative protein interaction assay in our lab, which we use to verify interactions between full-length proteins and predicted interfaces. This is achieved by designing and testing mutations that specifically disrupt the interface without perturbing the fold and stability of the protein, which can be monitored as part of the assay. We are now performing interaction tests in 96-well format at medium throughput and use our assay in collaborations with the labs of Julian König, Petra Beli and Sandra Schick (IMB).

***De novo* identification of protein interaction interfaces**
While it is important to have tools that identify instances of known types of interfaces in protein interactions, it is estimated that the majority of interaction interface types are still unknown. To advance the identification of new types of interfaces in protein interactions, we explore the use of crosslinking mass spectrometry (XL-MS). XL-MS has been demonstrated to provide interface information in case studies using purified proteins (or fragments of proteins). In collaboration with the Proteomics Core Facility of IMB, we are developing an experimental pipeline to perform XL-MS for interface mapping at medium throughput without the need for protein purification. We developed a positive and negative reference set of protein interactions, which we will use to benchmark our XL-MS method. We will then apply the method to protein interactions of interest to us and our collaborators.

Integrative systems biology
Integrating various omics data resources is a powerful strategy for deciphering the systems properties of cells and allows us to employ a data-driven approach to identify new cellular mechanisms. We integrate protein interaction, gene expression and mutation data with the aim of predicting the molecular mechanisms that mediate brain-specific phenotypes in neurodevelopmental disorders. We are also collaborating with the Schick lab to gain a systematic and global understanding of the role of BRG1- or BRM-associated factor (BAF) chromatin remodelling complexes in genome protection and repair.

FUTURE DIRECTIONS

Our focus will be to significantly advance the above research highlights to a point where we can apply them to study proteins and interactions that are implicated in neurodevelopmental disorders. Phase separation is an intriguing biophysical property of biopolymers such as proteins, and is often used to coordinate cellular function in response to stress. Protein interaction interfaces, especially those that involve disordered regions, play a critical role in the phase separation behaviour of proteins, and genetic mutations that perturb these interfaces have been linked to neurodegenerative disorders. We aim to apply our methods of protein interaction interface identification to study the mechanisms of phase separation that control protein homeostasis in the nucleus in collaboration with the lab of Petra Beli (IMB).

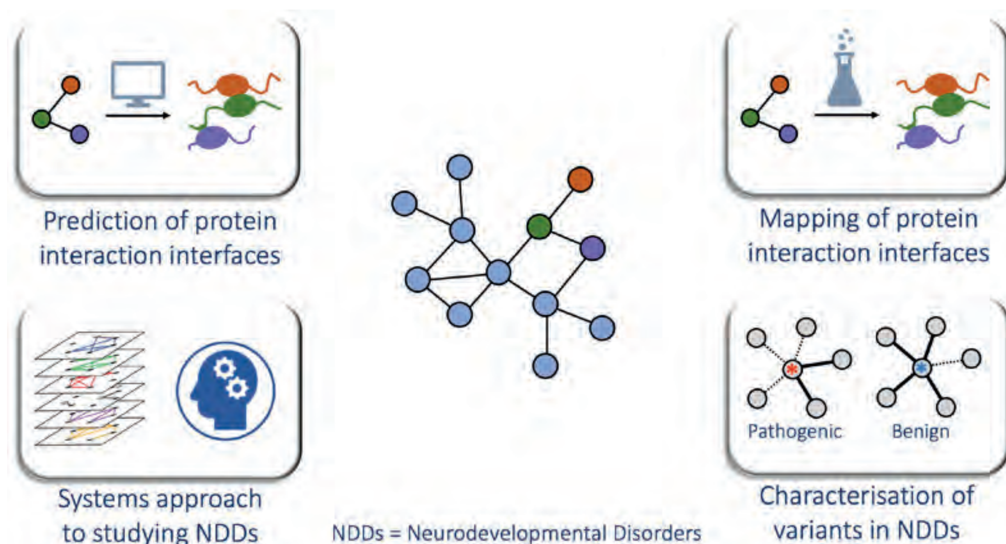

Figure 1. Interactions between proteins are central to cellular function. To improve our ability to predict phenotypes from genotypes, we develop computational and experimental methods to structurally and functionally characterise protein interactions, use interaction profiling to characterise mutations, and use omics data integration to predict cellular mechanisms within specific cellular contexts.

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Lab Manager; since 08/2020

SELECTED PUBLICATIONS

Luck K*, Kim DK*, Lambourne L*, Spirohn K* … Hill DE#, Vidal M#, Roth FP# and Calderwood MA# (2020) A reference map of the human binary protein interactome. *Nature*, 580:402–408

Kovács IA, Luck K, Spirohn K, Wang Y, Pollis C, Schlabach S, Bian W, Kim DK, Kishore N, Hao T, Calderwood MA, Vidal M and Barabási AL (2019) Network-based prediction of protein interactions. *Nat Commun*, 10:1240

Martínez-Noël G, Luck K, Kühnle S, Desbuleux A, Szajner P, Galligan JT, Rodriguez D, Zheng L, Boyland K, Leclere F, Zhong Q, Hill DE, Vidal M and Howley PM (2018) Network analysis of UBE3A/E6AP-associated proteins provides connections to several distinct cellular processes. *J Mol Biol*, 430:1024–1050

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BRIAN **LUKE**

OVERVIEW

R-loops are three-stranded structures consisting of an RNA-DNA hybrid and a displaced strand of ssDNA. They have previously been depicted as by-products of transcription that can lead to genomic instability, especially when confronted with the DNA replication machinery (Niehrs and Luke, 2020). Recently, it has become evident that R-loops also have important regulatory functions and can affect processes such as transcription, DNA repair, telomere protection and centromere function (Niehrs and Luke, 2020). Hence, it is important that R-loops are tightly regulated in a manner that allows them to be formed, while at the same time ensuring that they are efficiently removed to prevent the accumulation of "unscheduled" R-loops.

RNase H enzymes are important in preventing many human diseases

RNase H enzymes are conserved throughout evolution and promote cleavage of RNA that is base-paired to DNA. They are considered the major regulatory enzymes that control R-loop levels. RNase H2, which provides the majority of the RNase H activity in the cell, is a heterotrimer that removes R-loops, as well as single ribonucleotide insertions in dsDNA. Loss of RNase H2 leads to severe human diseases, including Aicardi Goutiers Syndrome and cancer. Understanding synthetic interactions will be important for the future treatment of such diseases.

RESEARCH HIGHLIGHTS

We have previously demonstrated that the E3 ubiquitin ligase Rtt101 interacts with the replisome to promote the repair of DNA lesions. Cells with deletions of any component of the Rtt101 complex show increased sensitivity to DNA damaging agents and have impaired growth when RNase H2 activity is compromised. Importantly, the growth defect in the absence of RNase H2 is caused by the loss of ribonucleoside monophosphate (rNMP) removal, and not by a failure to remove R-loops. We have demonstrated that when rNMPs accumulate, Rtt101 is required specifically in the S phase to promote cell survival. We hypothesised that when rNMPs are hydrolysed before S phase (resulting in a ss nick) they are converted into double-strand breaks upon DNA replication, and it is at this stage that Rtt101 is required to promote homology directed repair (HDR). Indeed, when we used cell cycle-regulated alleles of RNase H2 we were able to demonstrate that Rtt101 becomes essential when rNMPs are nicked in S phase. In order to look for genes that are also required for viability upon rNMP nicking in S phase, we performed and verified a genome-wide SGA (synthetic genetic array) screen to identify other genetic

POSITIONS HELD

Since 2017

Adjunct Director, Institute of Molecular Biology (IMB), Mainz

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Group Leader, Institute of Molecular Biology (IMB), Mainz

2009 – 2014

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EDUCATION

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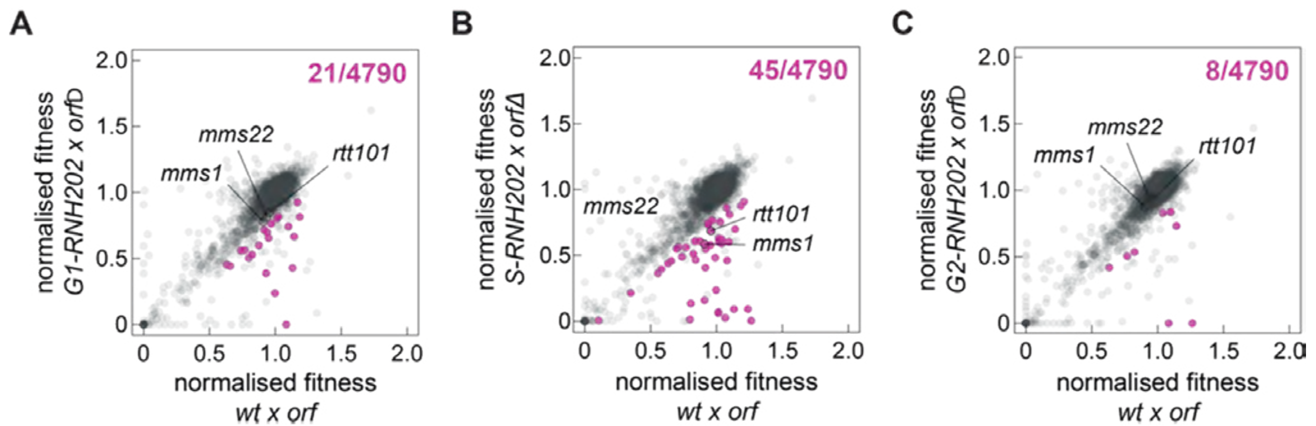

Figure 1. Using synthetic genetic arrays, we screened for mutants that had compromised viability when rNMPs were nicked in either G1 (A), S (B) or G2 (C) phase by expressing cell cycle restricted alleles of RNase H2 (RNH2). Along with Rtt101 and its interacting factor Mms1, many factors involved in HDR and histone deposition were identified as being required to deal with S phase nicks at rNMPs (analysis performed in collaboration with Anton Khmelinskii, IMB).

factors that, like Rtt101, are required for survival in the presence of S-phase nicks at rNMPs. We found that HDR factors and histone chaperones are both important for repairing nicks. Importantly, we were able to demonstrate epistatic genetic interactions between loss of *RAD51* and loss of *RTT101*, further supporting our initial hypothesis. In addition, we have identified relevant ubiquitin targets of Rtt101, including Dpb2 (a DNA polymerase subunit) and histone H3. Importantly, mutation of the ubiquitylation sites on H3 is able to phenocopy the effects of Rtt101 loss in the presence of high rNMP loads. In summary, our recent data shows that Rtt101-mediated ubiquitylation of histone H3 is required to tolerate rNMP accumulation in the absence of RNase H2. This project is funded by the CRC 1361 on "Regulation of DNA Repair and Genome Stability".

FUTURE DIRECTIONS

Accidentally inserted rNMPs are normally removed by RNase H2 in a reaction referred to as ribonucleotide excision repair (RER). When RNase H2 is mutated, as is frequently the case in chronic lymphatic leukaemia and prostate cancer, Top1 removes the rNMPs, which can in turn lead to 2-5 bp deletion mutations, especially in repetitive regions. We will use yeast genetics and mutagenesis assays to understand why Top1 only becomes mutagenic when RNase H2 is absent. This will be important in terms of cancer biology, as cells lacking RNase H2 show Top1-dependent genomic instability. Moreover, we will be verifying the synthetic interactions that we have found between *RTT101* and *RNASE H2* in human cells, to test whether this would be a viable avenue for therapeutic intervention. Finally, we will be trying to elucidate the mechanistic details of the HDR defect in *rtt101* cells when rNMPs accumulate.

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SELECTED PUBLICATIONS

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Lockhart A, Pires VB, Bento F, Kellner V, Luke-Glaser S and Luke B (2019) RNase H1 and RNase H2 are differentially regulated to process RNA-DNA hybrids. *Cell Rep*, 29:2890–2900.e5

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CHRISTOF **NIEHRS**

OVERVIEW

Although cellular DNA is commonly perceived as a static molecule that carries genetic information in the form of nucleotide sequences, genomic nucleobases are in fact physiologically modified by a variety of chemical modifications. For example, the recent discovery of three modified bases in mammalian DNA – 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) – was wholly surprising, and we still do not know if these bases are primarily DNA demethylation intermediates or whether they have epigenetic roles in their own right. These DNA modifications are deposited in the genome in a site-specific manner and are known or suspected to epigenetically regulate gene expression. Typically, DNA modifications are recognised by specific reader proteins and can be reversed by a variety of enzymatic mechanisms. We study which DNA modifications occur in the mammalian genome, how and where in the genome they are deposited, what biological role they play, and how they are recognised and removed. To reach this goal, we use ultrasensitive mass spectrometry to identify and quantify DNA modifications in mammalian cells. We employ next-generation sequencing and computational analysis to identify modification sites genome-wide. We characterise the roles of proteins involved in depositing, reading and removing modifications in embryonic stem cells, as well as in *Xenopus* embryos and mice.

We study chemical modifications that regulate genomic DNA

RESEARCH HIGHLIGHTS

DNA poly-ADP-ribosylation

Poly-ADP-ribosylation (PARylation) is a widespread post-translational modification of proteins where ADP-ribose from NAD+ is transferred to amino acid residues of target polypeptides. PARylation is catalysed by poly(ADP-ribose) polymerases (PARPs), which play important roles in many biological processes and diseases such as DNA repair and cancer. In mammalian cells, PARylation is regarded as a protein-specific modification. However, some PARPs were recently shown to modify DNA termini *in vitro*. These observations raise the intriguing possibility that PARylation is a novel DNA modification and pose the questions of whether mammalian DNA is PARylated, where in the genome the modification occurs, and what its physiological role could be.

To start addressing these questions, we probed PARylation of DNA by dot blot with an anti-poly ADP-ribose antibody. Total DNA from mouse embryonic stem cells (mESC) and human HEK293T cells were positive for PAR, while plasmid DNA from *E. coli* was negative (Figure 1a). We carefully

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EDUCATION

1997
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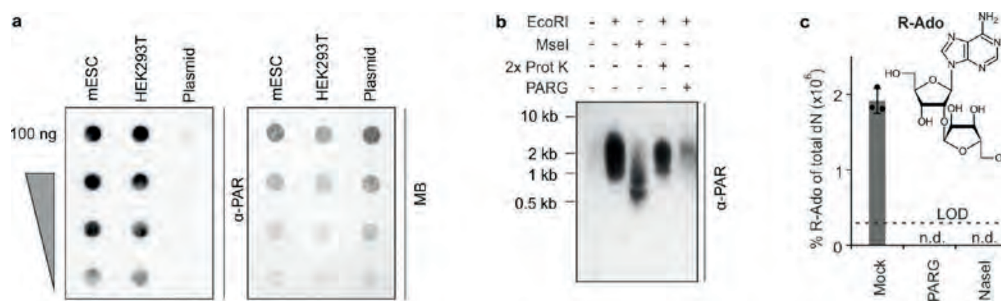

Figure 1. Poly-ADP-ribosylation occurs on mammalian DNA
A) Dot blot analysis for PARylation of mESC and HEK293T serially diluted genomic DNA. Plasmid DNA served as a negative control. MB, methylene blue staining. **B)** Southwestern blot analysis for PARylation of mESC DNA treated with EcoRI, MseI, proteinase K and PARG as indicated. Length of marker DNA is shown on the left. **C)** LC-MS/MS quantification of ribosyl-adenosine (R-Ado) on HEK293T DNA treated as indicated. Samples were repurified after enzyme treatments by a second column-based DNA purification to remove any PAR and DNA monomers (dashed line indicates limit of detection (LOD)).

validated the specificity of the antibody signal and ruled out both protein and RNA contamination. Treatment with PAR glycohydrolase (PARG), the primary enzyme responsible for degrading cellular ADP-ribose moieties, eliminated the PAR signal from mESC DNA, ruling out DNA cross-reactivity. Southwestern blot analysis of mESC DNA with anti-PAR antibody yielded a signal in form of a smear only after restriction digest, indicating that PAR chains are covalently attached to DNA, instead of free- or protein-bound PAR chains co-purifying during DNA preparation (Figure 1b). Analysis of DNA from diverse human organs revealed PARylation in all tested tissues but with notable differences, e.g. low levels in heart and muscle and high levels in brain, thymus, liver and placenta, suggesting biological specificity.

To independently confirm the mammalian DNA PARylation antibody results, we employed stable isotope dilution mass spectrometry (LC-MS/MS) to analyse ribosyl-adenosine (R-Ado), a product diagnostic for linear PAR chains. In DNA from HEK293T cells, R-Ado was detectable at very low levels ($2x10^{-6}$ % of total dN), corresponding to ~100 R-Ado monomers per genome (Figure 1c). R-Ado was also detected in DNA from mESCs and mouse organs, where notably liver DNA showed 10x higher levels than HEK293T cells. We calculated an average of 1-10 PARylation sites per genome in HEK293T cells.

Since protein PARylation is a hallmark of DNA repair and quickly occurs on sites of DNA damage, we asked if DNA damage is also accompanied by PARylation of DNA. Hence, we tested whether DNA PARylation localises to DNA double strand breaks (DSBs). We made use of DIvA cells as an experimental cell system. These cells harbour the DNA rare cutter AsiSI, which in presence of 4 hydroxytamoxifen (4-OHT) creates DSBs at defined positions across the genome and in various chromatin contexts. While 4-OHT treatment of DIvA cells robustly induced gH2AX nuclear foci, there was no induction of global DNA PARylation. To specifically monitor local changes in DNA PARylation, we analysed known AsiSI-mediated DNA break loci. While 4-OHT-induced gH2AX recruitment to these loci, PAR signals were low and remained unchanged by 4-OHT. We conclude that DNA PARylation is not a general feature of DNA breaks.

Taken together, our results reveal that PARylation is a rare, physiologically relevant DNA modification that is unrelated to DSB repair and occurs in primary mammalian tissues and cell lines.

FUTURE DIRECTIONS

Our results indicating PARylation is a physiologically relevant DNA modification in mammalian cells raise many questions. Which loci are PARylated? What is common to such loci? What determines the specificity of locus PARylation? What are the PARP enzymes involved? What is the physiological role of PARylation at these loci? Can we reconstitute DNA PARylation *in vitro*? To start addressing these questions, we will profile PARylation genome-wide by PAR-DIP-seq and use bioinformatics to analyse any peaks obtained.
In addition, we will establish *in vitro* PARylation assays using PARP enzymes and DNA oligonucleotides to characterise the reaction specificity and sites of PARylation and whether it occurs on a nucleobase or terminal ribose.

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SELECTED PUBLICATIONS

Musheev MU#, Baumgärtner A, Krebs L and Niehrs C# (2020) The origin of genomic N6-methyl-deoxyadenosine in mammalian cells. *Nat Chem Biol*, 16:630–634

Arab K, Karaulanov E, Musheev M, Trnka P, Schäfer A, Grummt I# and Niehrs C# (2019) GADD45A binds R-loops and recruits TET1 to CpG island promoters. *Nat Genet*, 51:217–223

Han D*, Schomacher L*#, Schüle KM*, Mallick M, Musheev MU, Karaulanov E, Krebs L, von Seggern A and Niehrs C# (2019) NEIL1 and NEIL2 DNA glycosylases protect neural crest development against mitochondrial oxidative stress. *eLife*, e49044

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VASSILIS ROUKOS

OVERVIEW

Maintaining the integrity of genetic information is essential for cell survival. Mechanisms that counteract DNA damage maintain cellular homeostasis by suppressing mutagenic events and genomic rearrangements that may lead to disease, particularly cancer. Chromosome translocations are one of the most severe forms of genomic rearrangements. Translocations form through the illegitimate joining of chromosome breaks and often play key roles in the initial steps of tumourigenesis. Despite their prevalence and importance, our understanding of their genesis is still rudimentary. Which molecular features define recurrent chromosome breakpoints? How do the broken chromosome ends find each other within the nuclear space? What are the DNA repair mechanisms that mediate chromosome fusion and which factors favour interchromosomal fusion (translocation) over intrachromosomal repair? By using a combination of molecular biology techniques, genetics and high-throughput imaging and sequencing approaches, we aim to shed light on the basic molecular mechanisms underlying the formation of oncogenic chromosome translocations.

We use genomics to understand why chromosomes break repeatedly at the same locations

RESEARCH HIGHLIGHTS

Novel imaging-based tools to probe rare, cancer-initiating genomic rearrangements

Modelling the formation of recurrent, cancer-initiating genomic rearrangements of interest requires a versatile approach that can probe rare events with high sensitivity. We have now established a method called CRI3D that uses fluorescence *in situ* hybridisation (FISH) to probe the position of individual chromosome ends of potential rearrangements in interphase cells in 3D. High-throughput microscopy and automated image analysis are then used to identify single cells with chromosome breakages and different rearrangements (deletions, inversions, fusions, etc.). This methodology complements existing approaches and offers several advantages: it is (i) suitable for detecting and quantifying rearrangements without the need for mapping the precise breakpoints or rearrangement product; (ii) compatible with both site-specific induction of breaks (mediated by endonucleases, ZNFs, CRISPR), as well as more physiological methods of inducing DNA damage, such as ionising radiation and chemotherapeutics (see below); and (iii) able to detect rearrangements in interphase cells at frequencies down to 10^{-4}

POSITIONS HELD

Since 2015

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EDUCATION

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MSc in Applications in Medical Sciences, University of Patras Medical School

without the need for metaphase spread preparation. CRI3D is a powerful tool that can be used to dissect molecular and cellular mechanisms that contribute to the formation of any oncogenic genome rearrangement of interest.

Mechanistic insights into the formation of therapy-related, oncogenic translocations

Cancers are commonly treated with anticancer drugs called topoisomerase poisons. Treatment with topoisomerase poisons, however, can also cause chromosome translocations in healthy cells that disrupt gene regulation and lead to the development of leukaemia. It is unclear why these leukaemia-promoting translocations are so common after treatment with topoisomerase poisons.

We are interested in combining cutting-edge genomics and single-cell imaging methods to determine why these leukaemia-promoting translocations arise. Our current work has shown that certain sites with highly active genes tend to be close to regions where DNA folds into chromatin loops and are under more mechanical strain. This makes them susceptible to DNA breaks caused by topoisomerase poisons such as etoposide, producing translocations that drive leukaemia (Figure 1). We have also identified factors involved in the repair of these DNA breaks that actively suppress the formation of translocations. In another direction that may have clinical implications, we are performing unbiased siRNA-based screens to identify factors that suppress these types of translocations while leaving the cytotoxic effect of topoisomerase poisons intact. Our findings highlight how gene activity and the arrangement of DNA within the nucleus can have a profound impact on events that trigger genomic instability to promote cancer.

FUTURE DIRECTIONS

Central to our focus is shedding light on the events that promote genomic instability in the context of chromatin and chromosome organisation. We therefore intend to profile endogenous DNA breaks across the genome in various cell types, with the aim of identifying common or cell type-specific signatures of DNA fragility. We will then focus on identifying mechanistically how these endogenous DNA breaks form and evaluate how DNA break repair efficiency is influenced by genomic and chromatin context. These studies will directly highlight the link between cell type-specific DNA fragility and repair in the formation of tissue-specific, recurrent oncogenic translocations. Taken together, our research will shed light on the mechanisms of cancer-initiating translocations, which will advance our knowledge of the fundamental principles of cancer aetiology.

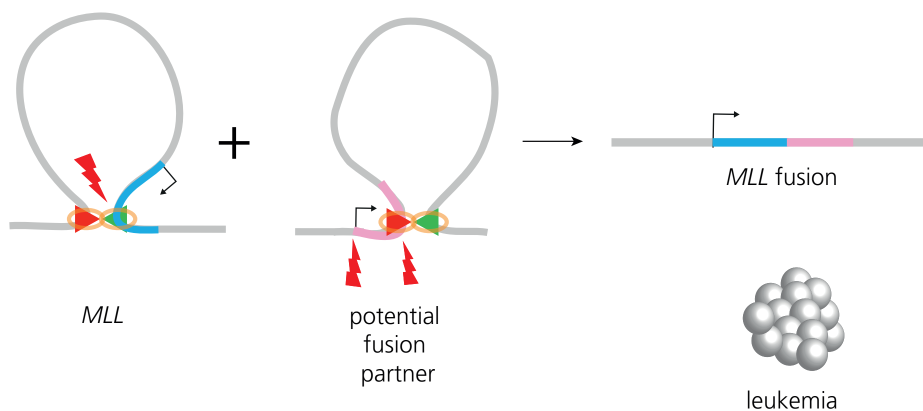

Figure 1. Etoposide-induced DNA double-strand breaks within highly transcribed *MLL* and fusion partner genes at loop boundaries promote the formation of oncogenic *MLL* translocations.

SELECTED PUBLICATIONS

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Roukos V (2018) Actin proteins assemble to protect the genome. *Nature*, 559: 35–37

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SANDRA **SCHICK**

OVERVIEW

Compaction of the genome into higher-order chromatin structures requires a variety of dynamic regulatory mechanisms for the temporal and spatial control of genomic processes. These regulatory mechanisms are particularly important for ensuring proper gene expression and thus the appropriate execution of all cellular processes. Various regulators act in an integrative and coordinated fashion, resulting in a highly complex and fine-tuned system. Therefore, it is not surprising that mutations in genes encoding these regulators are frequently associated with various diseases.

We study how mutations in genes that encode chromatin remodellers cause disease

In order to uncover how these regulators integrate and contribute to gene regulation, genome stability and other genomic processes, we employ mammalian – especially human – cellular model systems in combination with genome editing, epigenomics, proteomics and various molecular and biochemical approaches. Moreover, we explore the cellular and molecular consequences of mutations in these regulators in order to unravel the mechanisms underlying the associated diseases and identify potential therapeutic approaches.

RESEARCH HIGHLIGHTS

One class of chromatin regulators that is essential for modulating chromatin structure is the BRG1/BRM associated factor (BAF) chromatin remodellers. These remodellers are pleomorphic complexes comprised of multiple subunits that are encoded by more than 30 genes and assembled in a combinatorial fashion. There are three subtypes of BAF complexes, each with a few distinct subunits: the canonical BAF complexes (BAF/cBAF), the polybromo-associated BAF complexes (PBAF) and the non-canonical GLTSCR1/1L-BAF complexes (GBAF/ncBAF). These remodellers utilise energy from ATP hydrolysis to slide or eject nucleosomes and thereby modulate DNA accessibility. They control gene regulatory regions and consequently regulate a multitude of cellular functions, as well as developmental processes such as lineage specification and differentiation. Moreover, BAF complexes contribute to genomic processes such as the DNA damage response, DNA replication and sister chromatid cohesion, as well as chromatin topology and organisation. The unexpectedly high mutation rate in genes encoding various BAF subunits in cancer and neurodevelopmental disorders highlights the importance of these remodellers. Therefore, it is of key importance to elucidate the function of the diverse

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2012
MSc in Biomedicine and Diploma in Biology, Johannes Gutenberg University Mainz (JGU)

2008
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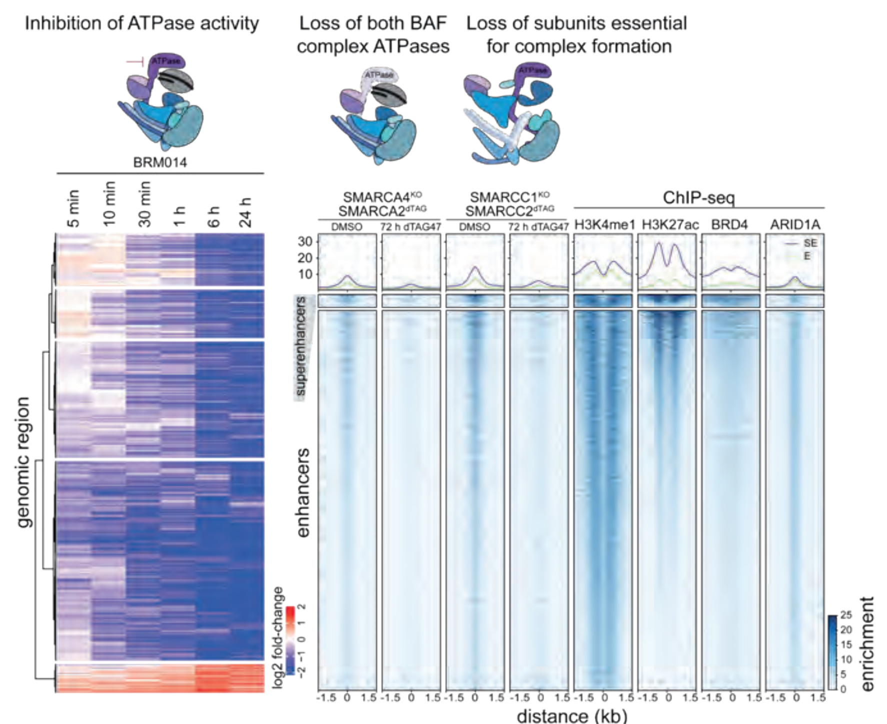
BAF complexes and the molecular consequences of mutations in genes encoding BAF complex subunits. These novel insights would likely enable the development of targeted therapeutics for BAF-associated diseases.

Cancer-associated loss-of-function mutations in genes encoding subunits of the BAF chromatin remodelling complexes often cause drastic chromatin accessibility changes, especially in important regulatory regions. Exploring this in an isogenic cell line panel comprising individual knockouts of most BAF-encoding genes illustrated that the alterations can vary depending on which subunit is lost. Additionally, the most profound effects were observed after knockout of the BAF genes that are most frequently mutated in cancer. Utilising the dTAG system to induce acute degradation of BAF subunits, we could further show that these alterations are mostly direct and immediate consequences of the lack of protein expression of the respective subunit. Moreover, pharmacological inhibition of BAF chromatin remodelling activity further demonstrated that maintaining genome accessibility requires constant ATP-dependent remodelling.

These studies also revealed dependencies between the different BAF subunits. In order to explore the vulnerabilities of the mutant cells to further BAF perturbations, a viability screen was performed to test the effects of concomitant loss of different BAF complex subunits in all possible combinations. This screen revealed several intra-complex synthetic lethalities that were further confirmed in additional cancer cell lines. Highly conserved synthetic lethalities were present between the two paralog ATPases required for remodelling activity and between two paralog subunits of the complex core, which are required for complex formation. Complete abrogation of BAF complex function by acute degradation of a synthetic lethal subunit in a paralog-deficient background results in almost complete loss of chromatin accessibility at BAF-controlled sites (especially at superenhancers), providing a potential mechanism for these intracomplex synthetic lethalities and a basis for therapeutic targeting of some BAF-mutant cancers.

Although these systematic studies performed at the CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences provide extensive new insights into the biology of BAF complexes and associated diseases, further research is needed to comprehensively understand the regulatory roles of different BAF subtypes in various cellular processes and different cellular backgrounds.

Figure 1. BAF chromatin remodelling activity is constantly required to maintain chromatin state. Perturbations that abolish BAF complex activity or formation result in immediate loss of chromatin accessibility at BAF-controlled enhancers and superenhancers, which is lethal for most cells. ATAC-seq results are shown unless indicated otherwise. SE = superenhancers, E = enhancers.



FUTURE DIRECTIONS

To accomplish this goal, we will further explore the molecular function and regulation of individual BAF subtypes. We will systematically investigate the processes they are involved in and how they integrate with other regulatory mechanisms. To study the role of individual BAF subtypes in various cell types, developmental processes and BAF mutation-induced diseases, we will use cell culture models closely reflecting human conditions. Ultimately, our research aims to unravel pathogenic mechanisms that can be targeted for therapy.

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SELECTED PUBLICATIONS

Schick S*#, Grosche S*, Kohl KE*, Drpic D, Jaeger MG, Marella NC, Imrichova H, Lin JMG, Hofstätter G, Schuster M, Rendeiro AF, Koren A, Petronczki M, Bock C, Müller AC, Winter GE and Kubicek S# (2021) Acute BAF perturbation causes immediate changes in chromatin accessibility. *Nat Genet*, 53:269–278

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LUKAS **STELZL**

OVERVIEW

Our aim is to elucidate how liquid-liquid phase separation and phase-separated condensates of proteins and nucleic acids provide specific regulation and how such specific regulation is lost in pathologies. We are a computational biophysics and computational biology group and use particle-based multi-scale simulations in our research.

The discovery that liquid-liquid phase separation and phase-separated condensates of proteins and nucleic acids are important regulators in cells is revolutionising our understanding of cell biology. Liquid-liquid phase separation of biomolecules is analogous to the demixing of oil and vinegar in a vinaigrette. Through liquid-liquid phase separation, biomolecules can form distinct liquid phases – a dilute phase and a protein- and nucleic acid-rich phase. Phase separation helps to organise biological functions in time and space. Thus, phase separation not only plays an important role in regulating genes at the transcriptional level, but also at the post-transcriptional level in developmental biology. Dysregulation of liquid-liquid phase separation is hypothesised to be an important driver of ageing and age-related diseases.

Computational modelling reveals how phase separation is dysregulated in neurodegeneration

RESEARCH HIGHLIGHTS

To understand the biological roles of liquid-liquid phase separation and phase-separated biomolecular condensates, we have developed multi-scale simulations of disordered proteins, their condensates and liquid-liquid phase separation. Simulations provide important insights into the conformational dynamics of biomolecules (Stelzl*, Erlenbach* *et al*, 2017) and their biomolecular function (Stelzl *et al*, 2020) and can thus complement experiments.

We have generated atomic-resolution structural ensembles of disordered proteins and their phase-separated condensates. To do this, we developed a hierarchical approach. Standard atomistic simulations of such systems are fraught with difficulty, as disordered proteins can adopt many different conformations. In our hierarchical simulation method, we efficiently explored the possible chain conformations of disordered proteins while resolving their local structure at atomic resolution. As a first step, we benchmarked computational models for α-synuclein, which on its own and in its monomeric form is often used as a model biophysical system for disordered proteins. Our model showed very good agreement with NMR data on α-synuclein's local structure, as well as its

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EDUCATION

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average global structural characteristics as probed by small-angle X-ray scattering (SAXS) (Pietrek*, Stelzl* *et al*, 2020). Next, we performed atomistic modelling for disordered tau, which is critical in Alzheimer's disease, and found not only agreement with many different experimental datasets, but importantly we also resolved biologically relevant conformations in our highly diverse and representative ensemble of tau (Stelzl* & Pietrek*, *bioRvix*, 2021). We found that the two aggregation-prone hexapeptide motifs sample conformations, similar to tau fibrils from patient samples. These two hexapeptide motifs flank a PGGG motif. P301 to L/S/T mutations give rise to devastating disease and are used in mouse models of Alzheimer's disease. These mutations shift the conformational equilibrium of this turn towards extended structures. Turns between the two aggregation-prone hexapeptide motifs may be essential for preventing the formation of extended beta-strands and pathological aggregation. Our ensemble agrees with biophysical (e.g. NMR) data on local and long-range structure. We demonstrated that with the same hierarchical approach, we can also simulate phase-separated condensates of disordered proteins (*in preparation*).

Phosphorylation of TDP-43 is a hallmark of neurodegenerative disease and the paradigm of the field has been that phosphorylation induces liquid-liquid phase separation and pathological aggregation of TDP-43. Experiments by the laboratory of Dorothee Dormann (IMB/JGU) have questioned this prevailing dogma and offered a new interpretation: her research has shown that phosphorylation and phosphomicking mutations actually reduce propensity to phase separation and aggregation. We have started to complement these experiments with multi-scale simulations (Gruijs da Silva *et al*, *bioRvix*, 2021). Using multi-scale simulation methods that we developed (Pietrek*, Stelzl* *et al*, 2020), we showed that phosphomicking S->D mutations favours increased solvation of TDP-43 and thus provided a mechanistic basis for the proposed cyto-protective effects (Figure 1). Moreover, we showed that hyper-phosphorylation dissolves TDP-43 condensates, in line with the cyto-protective role of phosphorylation proposed by Dorothee Dormann. Our simulations also revealed that aromatic residues engage in "sticker-sticker" interactions in accordance with the "sticker-spacer" model, and thus our study provides a first step towards understanding such interactions on the molecular scale with atomic resolution.

FUTURE DIRECTIONS

We want to understand disease-linked mutations and modifications on a much larger scale. In collaboration with the Dormann lab (IMB /JGU), we will simulate large numbers of neurodegeneration-linked post-translational modifications and mutations to better understand how they modulate phase behaviour and aggregation in disease.

We are increasingly dealing with complex multi-component condensates in our simulations to understand gene regulation. It will be particularly exciting to investigate multi-component protein-RNA condensates in post-transcriptional gene regulation in collaboration with the Ketting lab (IMB).

We will also continue to further develop our simulation methods to improve our models and better match experimental complexity.

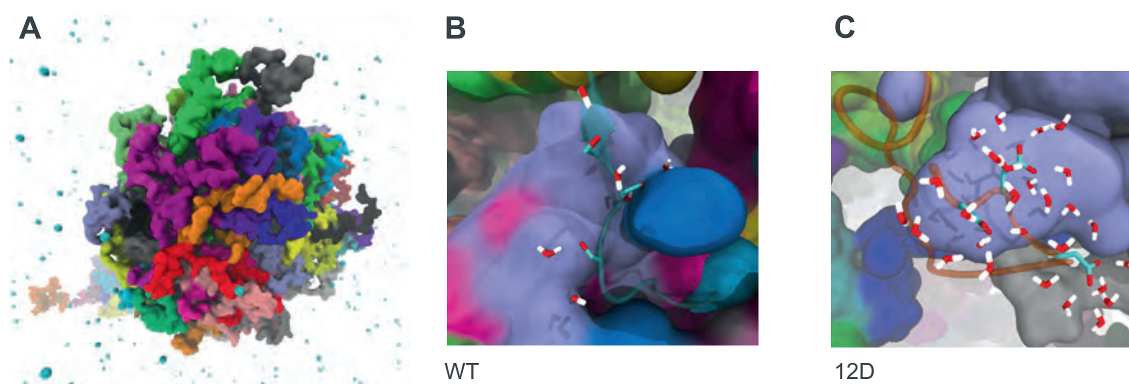

Figure 1. Figure 1. Multi-scale simulations of TDP-43. A) Phase-separated TDP-43 condensate. B) In WT TDP-43, serine residues at the site of the phosphomimicking mutations Ser->Asp interact with other protein residues and with water. C) Aspartate residues at sites of the phosphomimicking mutations Ser->Asp lose many protein-protein interactions, gain only a few charge-charge interactions and interact primarily with water.

SELECTED PUBLICATIONS

Pietrek LM*, Stelzl LS* and Hummer G (2020) Hierarchical ensembles of intrinsically disordered proteins at atomic resolution in molecular dynamics simulations. *J Chem Theory Comput*, 16:725–737

Stelzl LS, Mavridou DAI, Saridakis E, Gonzalez D, Baldwin AJ, Ferguson SJ, Sansom MSP and Redfield C (2020) Local frustration determines loop opening during the catalytic cycle of an oxidoreductase. *elife*, 9:e5466

Stelzl LS*, Erlenbach N*, Heinz M, Prisner TF and Hummer G (2017) Resolving the conformational dynamics of DNA with Ångstrom resolution by pulsed electron – electron double resonance and molecular dynamics. *J Am Chem Soc*, 139:11674–11677

*indicates joint contribution, #indicates joint correspondence

GROUP MEMBERS

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Intern; since 07/2021

Arya Changiarath Sivadasan
PhD Student; since 04/2021

HELLE **ULRICH**

OVERVIEW

A robust response to DNA replication stress is an important defence mechanism against genome instability and serves as a last barrier against cancer. Our lab studies regulatory mechanisms that contribute to ensuring the complete and accurate duplication of a cell's genetic information, especially as they relate to the posttranslational protein modifiers ubiquitin and SUMO. We aim to understand how cells choose between alternative processing pathways for replication-blocking lesions in the DNA template, e.g. between error-prone translesion synthesis and accurate recombination-mediated template switching, or between fork-associated and postreplicative modes of damage bypass. Posttranslational modifications of the replication clamp protein PCNA have proven to be critical determinants of these pathways in eukaryotes. To elucidate the spatial and temporal regulation of ubiquitin-dependent DNA damage bypass and other cellular processes controlled by the ubiquitin family, we generated a series of affinity probes for use as biosensors in fluorescence microscopy. Characterisation and application of such probes can provide insight into the relevant pathways at the single-cell level, thus providing a high-resolution picture of posttranslational modifications in the cell.

> *"We use affinity probes to provide insight into ubiquitin- and SUMO-regulated pathways"*

RESEARCH HIGHLIGHTS

Antibodies and affinity probes are widely available for detecting posttranslational modifiers of the ubiquitin family. However, many can only be used *in vitro* and most act as inhibitors because they interfere with the modifiers' interactions with their physiological partners. In collaboration with Andreas Plückthun (University of Zurich), we used ankyrin repeat proteins (DARPins) as a framework to select affinity probes for yeast and human SUMO, as well as for the diubiquitin units of distinct linkage K48 and K63. At moderate expression levels, these probes can be used to track the respective posttranslational modifications in cells. This revealed that DNA damage-induced nuclear foci of SUMO overlap with regions of ssDNA in budding yeast and an association of both K48- and K63-linked polyubiquitin chains with cytoplasmic stress granules. The linkage-selective anti-ubiquitin DARPins are particularly promising as useful tools because they – unlike commercially available probes – exhibit high affinity even for short chains. Moreover, we isolated a K48-specific DARPin that does not appear to interfere with conjugation, deconjugation or cellular ubiquitin metabolism.

In collaboration with Robert Cohen (Colorado State University), we developed a series of probes against ubiquitylated PCNA as a direct marker of DNA damage bypass. Composed of a

POSITIONS HELD

Since 2013

Scientific Director, Institute of Molecular Biology (IMB), Mainz

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2018 – 2019

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2004 – 2012

Group Leader, Clare Hall Laboratories, Cancer Research UK London Research Institute

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Group Leader, Max Planck Institute for Terrestrial Microbiology, Marburg

1998 – 2000

Postdoc, Max Planck Institute for Biochemistry, Martinsried

1997 – 1998

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EDUCATION

2004

Habilitation in Genetics, Philipps University Marburg

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PhD in Chemistry, University of California, Berkeley

1994

Diploma in Biology, Georg August University Göttingen

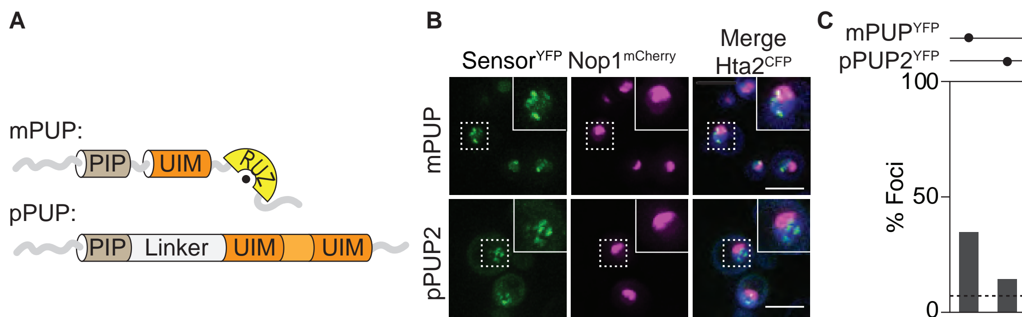

Figure 1. Ubiquitin- and SUMO-specific biosensors. A) Probes against total ubiquitylated (mPUP) and polyubiquitylated PCNA (pPUP). PIP: PCNA-interacting peptide; UIM, RUZ: ubiquitin-binding domains. **B)** Enrichment of ubiquitylated PCNA in nucleoli, marked by Nop1^{mCherry}. Nuclei are marked by CFP-tagged histone H2A (Hta2). Scale bar = 5µm **C)** Quantification of the nucleolar portion of ubiquitylated PCNA. The dashed line indicates the expected random distribution.

PCNA-interacting peptide and a set of ubiquitin-binding domains with varying affinities, these probes allow us to differentiate between total ubiquitylated and specifically polyubiquitylated PCNA (Figure 1A). While at high expression levels these probes sensitise yeast cells to DNA-damaging agents, they can be used as biosensors when expressed at moderate levels. Having validated their target specificities *in vivo*, we used them to monitor the distribution of ubiquitylated PCNA relative to markers of DNA replication and replication stress. DNA damage induced distinct foci of ubiquitylated PCNA with a timing similar to, but earlier than, the ssDNA-binding RPA complex and with increasing overlap over the course of S phase. Compared to RPA foci, which represent postreplicative daughter-strand gaps and thus the substrates of damage bypass, a stronger overlap was observed between the probes and the replicative MCM helicase during early S phase. We conclude that PCNA ubiquitylation occurs in close proximity to replication forks and precedes the expansion of daughter-strand gaps but persists even when these gaps have formed larger postreplicative repair territories. Remarkably, we observed a relative enrichment of total ubiquitylated PCNA in the nucleolus, possibly reflecting the problematic nature of the rDNA as a replication template (Figure 1B-C). In contrast, polyubiquitylated PCNA – a marker for the template switching pathway – was not strongly enriched, consistent with the notion that recombination events in the rDNA proceed preferentially outside the nucleolus. Thus, our biosensors for ubiquitylated PCNA have allowed us to gain detailed insights into its nuclear distribution and choreography.

FUTURE DIRECTIONS

The compact framework of the DARPins is highly suitable for use in combination with other domains. Hence, we envision developing affinity probes selective for specific ubiquitylated or sumoylated substrates, similar to the development of probes against ubiquitylated PCNA. Moreover, a combination of K48- and K63-selective anti-ubiquitin DARPins could be exploited as a biosensor for polyubiquitin chain branching, thus advancing our understanding of this understudied phenomenon. Affinity purification of short chains using ubiquitin-selective DARPins might also facilitate the identification of additional ubiquitylation substrates that have eluded capture by commercial probes. Finally, we are developing sensors directed against ubiquitylated PCNA to explore its genome-wide distribution. Attempts at chromatin immunoprecipitation have so far failed, but fusion to a nuclease domain according to the 'cut-and-run' technology appears promising. Fusion to a biotin ligase domain will in turn enable the use of proximity labelling to identify relevant interaction partners.

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SELECTED PUBLICATIONS

Sriramachandran AM, Petrosino G, Méndez-Lago M, Schäfer AJ, Batista-Nascimento LS, Zilio N[#] and Ulrich HD[#] (2020) Genome-wide nucleotide-resolution mapping of DNA replication patterns, single-strand breaks, and lesions by GLOE-Seq. *Mol Cell*, 78:975–985.e7

Wong RP, García-Rodríguez N, Zilio N, Hanulová M and Ulrich HD (2020) Processing of DNA polymerase-blocking lesions during genome replication is spatially and temporally segregated from replication forks. *Mol Cell*, 77:3–16.e4

García-Rodríguez N, Morawska M, Wong RP, Daigaku Y and Ulrich HD (2018) Spatial separation between replisome- and template-induced replication stress signaling. *EMBO J*, 37:e98369

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EVA **WOLF**

OVERVIEW

Circadian clocks – operated by cell-autonomous transcription/translation feedback loops – affect many essential cellular, physiological and behavioural processes such as the sleep-wake cycle, hormone production, metabolism and the immune system. In mammals, the BMAL1/CLOCK transcription factor complex activates three period (*per1,2,3*) and two cryptochrome (*cry1,2*) clock genes, as well as many clock-controlled genes (ccgs), in a day-time dependent manner. Genome-wide analyses revealed three temporally separated phases of BMAL1/CLOCK-dependent circadian gene regulation (Figure 1): (1) a transcriptionally active state, where BMAL1/CLOCK recruits co-activators such as the histone acetyltransferase CREB-binding protein (CBP) and the histone methyltransferase Mixed Lineage Leukemia 1 (MLL1), (2) an early repressive state, where BMAL1/CLOCK is repressed by a large multi-subunit complex containing CRY/PER, as well as chromatin modifiers and RNA-binding proteins, and (3) a late repressive state, where CRY1 alone represses BMAL1/CLOCK. To further our mechanistic understanding of the transcriptional and epigenetic regulation of the mammalian circadian clock and ccgs, we pursue structure-function analyses of clock protein interactions with coactivators or co-repressors of BMAL1/CLOCK.

We study the protein interactions that keep the circadian clock running

RESEARCH HIGHLIGHTS

We have investigated the role of WD repeat-containing protein 5 (WDR5), a known component of the MLL1 complex, in the mammalian circadian clock. The MLL1 histone methyltransferase complex is recruited to BMAL1/CLOCK in a circadian manner to co-activate BMAL1/CLOCK by methylating histone H3 K4, thereby generating open chromatin. The active MLL1 complex includes the proteins WDR5, MLL1, RbBP5, Ash2L and DPY30, where MLL1 and RbBP5 bind to two different binding pockets of WDR5. Interestingly, WDR5 was also identified within a repressive PER-containing clock protein complex. Furthermore, WDR5 was shown to interact with PER1 and PER2 in cell-based co-IP studies. Hence, WDR5 may affect both the active state (via MLL1) as well as the early repressive state (via PER) of BMAL1/CLOCK to regulate circadian genes. Additionally, WDR5 deletion eliminates the circadian changes caused by activating and repressing histone methylation marks. We propose that WDR5 and its interactions with MLL1 or PER play a role in transitioning from the active- to early repressed BMAL1/CLOCK complex, and that this correlates with a circadian transition from open (active) to closed (repressive) chromatin states.

To elucidate the roles of WDR5 and PER-WDR5 interactions in the circadian clock, we set

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Since 2013
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2009 – 2011
Group Leader, Max Planck Institute for Biochemistry, Martinsried

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Group Leader, Max Planck Institute for Molecular Physiology, Dortmund

1996 – 2000
Postdoc, Rockefeller University, New York

EDUCATION

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Habilitation in Biochemistry, Ruhr University, Bochum

1996
PhD in Biology, European Molecular Biology Laboratory (EMBL), Heidelberg

1991
Diploma in Biology, University of Heidelberg

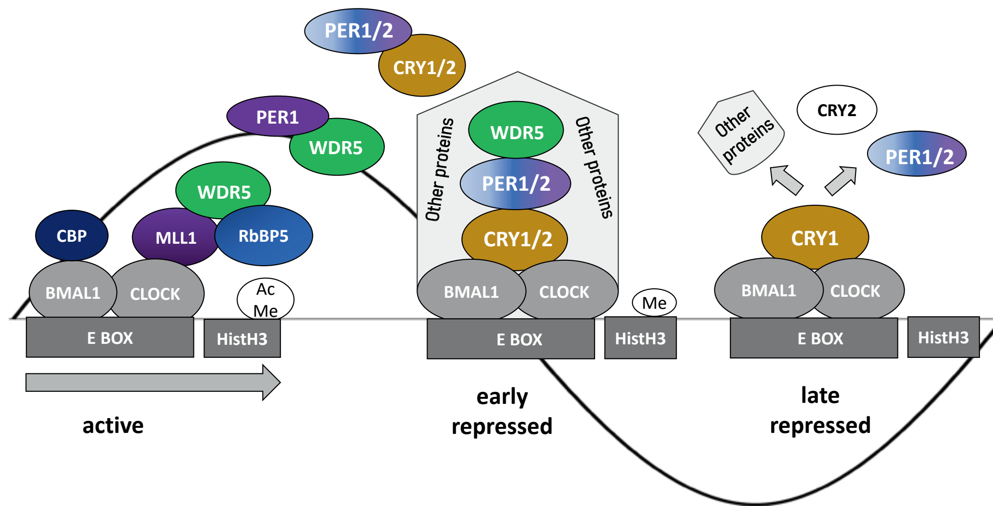

Figure 1. Day-time dependent transcriptional regulation of the mammalian circadian clock. **Left:** MLL1 (within the MLL1-WDR5-RbBP5-Ash2L-DPY30 complex) and CBP co-activate BMAL1/CLOCK. **Middle:** BMAL1/CLOCK is repressed by a multi-subunit "early repressive complex" including CRY1/2, PER1/2, WDR5, and about 30 other proteins. PER can interact with CRY or WDR5. **Right:** Late repressive CRY1/BMAL1/CLOCK complex. PER-WDR5 complexes may regulate the transition from active to early repressed BMAL1/CLOCK.

out to structurally, biochemically and biophysically analyse PER1/2-WDR5 interactions and their relationship to repressive CRY-PER and activating MLL1-WDR5-RbBP5 interactions. Interestingly, we found that PER1 and PER2 interact with WDR5 in different ways, resulting in distinct interplays with the formation of the PER-CRY clock protein complex and with WDR5-RbBP5 interactions. We suggest that PER1, which is known to arrive at circadian promotors before PER2, has a role in terminating the active state of BMAL1/CLOCK by MLL1 inactivation. A molecular switch from PER-WDR5 to later-occurring PER-CRY interactions would subsequently initiate assembly of the early repressive complex (Figure 1).

Our investigations have provided new insights into the partially non-redundant roles of PER1 and PER2 in the mammalian circadian clock. Additionally, they advance our understanding of the architecture and assembly of the early repressive BMAL1/CLOCK complex. They also illustrate how protein interactions that change daily can temporally regulate the transition from the active- to early repressive BMAL1/CLOCK complex and thereby define the circa 24 hour period of the mammalian circadian oscillator.

However, a lot of open questions remain, such as: are PER-WDR5 complexes incorporated into the early repressive complex, or do they only occur at the transition between the active and early repressed state? If the former is true, how do PER-WDR5 and PER-CRY sub-complexes integrate into the interaction network of the multi-subunit early repressive complex? Do PER-WDR5 complexes also have functions outside the circadian clock? Do PER-WDR5 interactions impact MLL1 histone methyltransferase activity (e.g. upon BMAL1/CLOCK co-activation) or the activity of other WDR5-containing cellular complexes? How are PER-WDR5 interactions regulated in the cell? Answering these questions will require much more extensive studies of the interaction networks of circadian clock proteins.

FUTURE DIRECTIONS

With the structural, biochemical and biophysical characterisation of WDR5-PER1/2 interactions, we have expanded our mechanistic understanding of the interaction network driving gene regulation in the mammalian circadian clock. Our studies identify mutants and interaction interfaces that can be used to dissect the roles of PER1/2-WDR5 interactions in the mammalian circadian clock in a targeted manner. They also provide a stepping stone to further reconstruct the architecture of multi-subunit clock protein complexes. Furthermore, the WDR5-PER interaction connects the core clock machinery to other epigenetic and gene regulatory processes in which WDR5 plays a role. In future, we will continue to structurally, biochemically and biophysically analyse new clock protein ligands and place them in the context of our existing knowledge of clock protein interactions.

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PhD Student; since 09/2021

SELECTED PUBLICATIONS

Li H*, Kilgallen AB*, Münzel T, Wolf E, Lecour S, Schulz R, Daiber A# and Van Laake LW# (2020) Influence of mental stress and environmental toxins on circadian clocks - implications for redox regulation of the heart and cardioprotection. *Br J Pharmacol*, 177: 5393–5412

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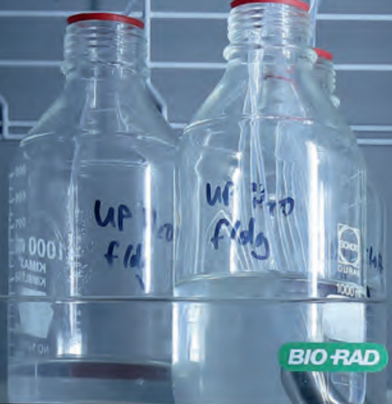
*indicates joint contribution, #indicates joint correspondence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OVERVIEW

The Core Facilities provide access to key technologies, as well as services & training by experts

IMB has seven Core Facilities (CFs): Bioinformatics, Flow Cytometry, Genomics, Microscopy/Histology, Proteomics, Protein Production and a Media Lab. The Bioinformatics, Genomics and Proteomics CFs provide a "full service", encompassing experimental design and quality control to data generation, analysis and data presentation. The Flow Cytometry and Microscopy/Histology CFs provide an "assisted service", where researchers work independently on CF equipment after introductory training by CF staff. The CFs' staff are available for consultation and troubleshooting for all users, whether they receive a full or assisted service. Furthermore, the CF staff can collaborate with researchers to provide customised or specialised services. IMB researchers have access to all seven CFs. In addition, the Flow Cytometry, Genomics, Microscopy/Histology and Proteomics CFs are open to external users from the larger research community in Mainz.

CF services are adjusted based on user demand. Each facility has a user committee that provides feedback on the equipment and user experience. This feedback also helps determine the implementation of new CF services. The overall CF functions as a service axis by aligning and combining individual services within its units to create new, innovative workflows (e.g. single cell sequencing, which requires a service overlap between Flow Cytometry and Genomics). The CFs also offer lectures and practical courses on new techniques and instrumentation, data acquisition, experimental design, statistics and data processing and analysis to allow researchers to keep up-to-date with current and emerging technologies. Lectures are generally open to everyone, including those outside of IMB.

IMB's CFs also maintain and provide training for IMB's core equipment and are responsible for managing the radioactivity lab, the S2 lab and IMB's in-house animal facilities (mouse, zebrafish and *Xenopus*).

In 2020, the CF hired a biostatistician to assist researchers with biostatistics analysis. This biostatistician is part of IMB's Compliance Board, which includes other representatives from management and staff, and makes recommendations to IMB's Scientific Directors on compliance and safety issues.

Furthermore, the CFs are responsible for institute-wide aspects of occupational health and safety, including all safety measures relating to the COVID-19 pandemic. The CF offers all IMB employees a coronavirus qPCR test twice a week so that IMB can remain a safe place to work.

Andreas Vonderheit
Director of Core Facilities and Technology

BIOINFORMATICS

OVERVIEW

The Bioinformatics Core Facility (BCF) supports IMB researchers with computing infrastructure, web services, system administration, software training, experimental design, biostatistics and data analysis. BCF members also participate in the computational processing, analysis, visualisation and interpretation of high-throughput data generated in research projects. BCF strives to offer tailored solutions for next-generation sequencing (NGS) and other omics methods. The BCF also supports the Collaborative Research Center on "Regulation of DNA Repair & Genome Stability" (CRC 1361) and the "Science of Healthy Ageing Research Programme" (SHARP).

SERVICES OFFERED

BCF staff offer support depending on the project needs, ranging from basic IT and bioinformatics services to full-scale scientific collaborations. Services include:

- Consulting on biostatistics and experimental design of genomics projects
- Data quality assessment, processing, analysis, visualisation and interpretation
- Implementation of NGS pipelines and customising them for individual projects
- Development of novel software tools and custom methods for specific analysis tasks
- Data mining of published datasets, correlation and integration of results
- Assistance with preparing manuscripts, presentations and grant proposals
- Workshops and tutorials to facilitate data access and analysis
- Basic biostatistics course for all IPP students (open to all IMB employees)
- Testing, implementation and customisation of software tools and online services
- System administration and IT support in cooperation with the JGU Data Centre

Throughout the years, the BCF has maintained and extended the computational and storage infrastructure of IMB. We maintain GitLab and GitHub repositories (https://gitlab.rlp.net/imbforge & https://github.com/imbforge) with software tools and pipelines for advanced NGS data analysis. In addition to standard tools and pipelines, the BCF offers customised bioinformatics solutions and long-term analytical support for data-intensive omics projects that require expert handling.

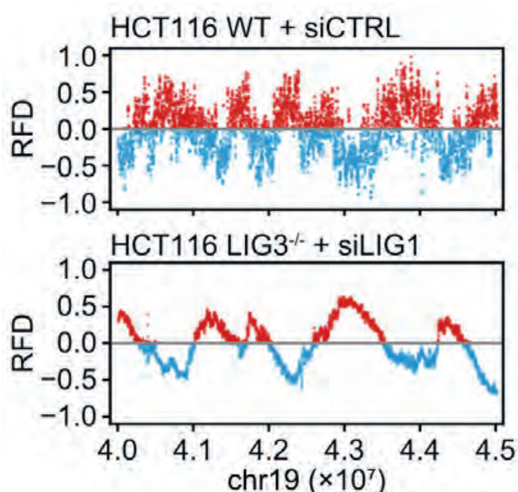

Figure 1. Strand break patterns associated with DNA replication in ligase-competent (WT) versus ligase-deficient HCT116 cells as detected by GLOE-seq, a novel analytical method developed in the context of CRC 1361 and published in Sriramachandran *et al*, 2020 (doi: 10.1016/j.molcel.2020.03.027).

CORE FACILITY MEMBERS

Emil Karaulanov
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Bioinformatician; since 01/2014

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System Administrator; since 04/2017

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Bioinformatician; since 03/2017

Frank Rühle
Bioinformatician; since 01/2019

Sergi Sayols
Bioinformatician; since 06/2019

Pascal Silberhorn
System Administrator; since 12/2015

FLOW CYTOMETRY

OVERVIEW

The Flow Cytometry Core Facility (FCCF) offers services for high-throughput measurements and analysis and separation of biological units through four different systems: a large particle sorter, a cell sorter and two analysers. With this equipment, the FCCF can analyse and sort particles of 0.5 µm to 1,000 µm in diameter.

CORE FACILITY MEMBERS

Stefanie Möckel
Head; since 10/2016

Stephanie Nick
Staff Scientist; since 01/2021

SERVICES OFFERED

The FCCF offers a full service for sorting, as well as an assisted service with training for the analysers. Additionally, its staff are available for collaborations to analyse flow cytometry data and prepare samples. During the past year, the FCCF has performed various types of experiments including multicolour measurements, cell separation for next generation sequencing, sorting of isolated neuronal nuclei, classical enrichments for subsequent cell culture, qPCR analysis, mass spectrometry and microscopy. The FCCF works with many different types of material, including nuclei, stem cells, yeast, *C. elegans*, *Arabidopsis* seeds, autophagosomes and lipid droplets, as well as various cultured cell lines and primary cells from humans, mice, zebrafish and *Drosophila*. To educate and train users, the FCCF offers three different lectures and an annual practical course for basic flow cytometry analysis, as well as an advanced practical course for cell sorting. In October, the FCCF laboratories moved into new rooms to enable the sorting of cells and organisms under BSL-2 conditions.

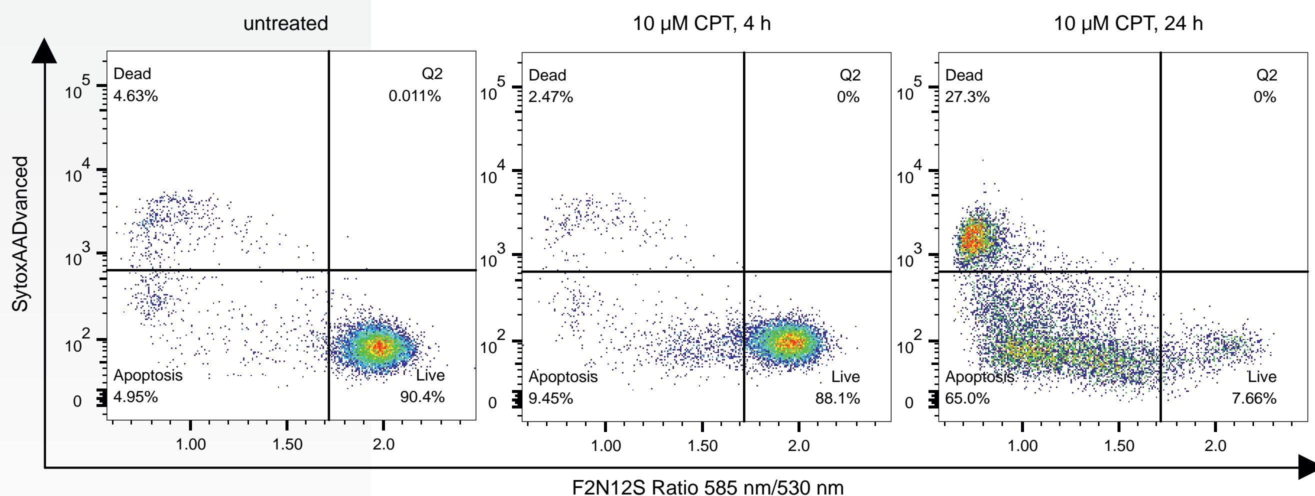

Figure 1. Apoptosis of adherent cells as measured by membrane asymmetry.

HeLa cells were left untreated or treated with 10 µM camptothecin (CPT) for 4 and 24 hours. Cells were labelled with Violet Ratiometric Membrane Asymmetry Probe (F2N12S) and SytoxAADvanced for labelling of dead cells and analysed by flow cytometry using the BD LSRFortessa. Apoptotic cells can be identified by a decreased ratio of the F2N12S probe.

GENOMICS

OVERVIEW

The Genomics Core Facility (GCF) offers next-generation sequencing (NGS) services based on the Illumina NextSeq500 and MiSeq platforms. A MinION sequencer (Oxford Nanopore Technologies) is also available. Standard library preparation services are offered, or users can submit their own prepared libraries for sequencing. The GCF also collaborates with researchers to develop new sequencing methods and pipelines.

SERVICES OFFERED

The GCF provides a full service for NGS, beginning with the experimental design of the project and continuing to the generation of sequencing data. In addition, the GCF also sequences self-prepared libraries from researchers at IMB, Mainz University and the University Medical Center.

After submission of RNA or DNA samples, the GCF performs initial quality control, library preparation, quality control of the prepared libraries, sequencing and raw data generation. Currently, the GCF supports library preparation for more than 20 applications as a standard service. New protocols are also developed to accommodate the user's needs for their specific projects. Protocols that the GCF supports include:

RNA:
- Strand-specific mRNA-Seq with poly-A selection
- Strand-specific total RNA-Seq with rRNA depletion
- Low input RNA-Seq
- Small RNA-Seq
- RIP-Seq
- Bru-Seq
- cDNA library preparation
- circRNA
- GRO-Seq
- STARR-Seq
- 3' Quant-seq
- Single-cell Gene Expression (10x Genomics) and SmartSeq2 protocols
- Single-cell Multiome ATAC & GE (10x Genomics)

DNA:
- ChIP-Seq
- MBD-Seq
- Whole genome sequencing
- Whole genome bisulfite sequencing
- Single-stranded DNA library preparation
- Hi-C
- MeDIP
- 8-oxoG
- DamID

User-prepared libraries:
- iCLIP-Seq
- Amplicon-Seq
- ATAC-Seq
- 4C / Capture-C
- RR-MAB-Seq
- GLOE-Seq
- LAM-HGTS

CORE FACILITY MEMBERS

Maria Mendez-Lago
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Annabelle Dold
Staff Scientist; since 01/2020

Pablo Llavona
Staff Scientist; since 11/2021

Hanna Lukas
Technician; since 01/2013

Regina Zimmer
Technician; since 12/2019

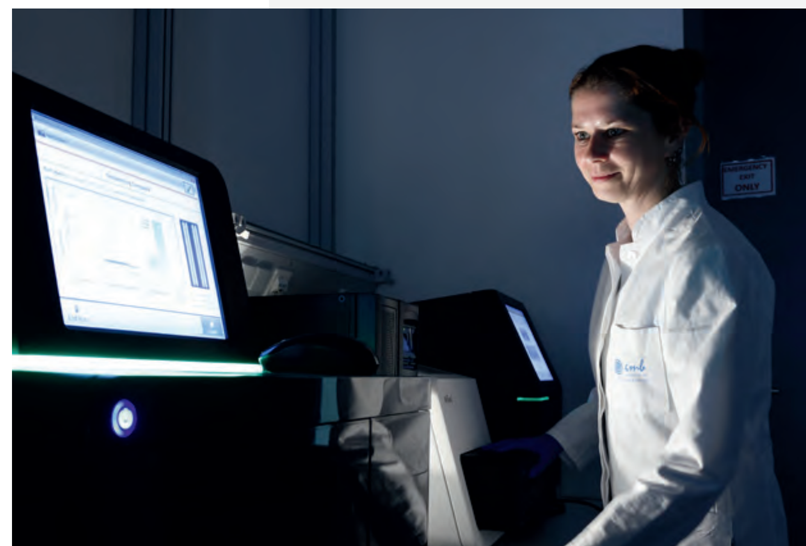

Figure 1. NextSeq 500 benchtop high throughput sequencer from Illumina

MICROSCOPY & HISTOLOGY

OVERVIEW

The Microscopy and Histology Core Facility (MHCF) provides users with a range of state-of-the-art microscopes and histology instruments, as well as expert guidance in sample preparation and data post-processing. Users benefit from a broad range of lectures and hands-on training and can choose from an independent, assisted or full service.

CORE FACILITY MEMBERS

Sandra Ritz
Head; since 01/2016

Márton Gelléri
Staff Scientist; since 06/2019

Petri Turunen
Staff Scientist; since 08/2019

SERVICES OFFERED

The MHCF offers 13 instruments, ranging from stereo and widefield microscopes to confocal, high-content screening and super-resolution microscopes. Seven set-ups (three widefield, one holotomography, one scanning confocal and two spinning disk confocal microscopes) are equipped for live-cell imaging. In 2021, the MHCF integrated three new live-cell set-ups: an ultra-modern widefield system (Thunder, Leica) with deconvolution options, a holotomography system for label-free imaging (3D cell explorer CX-F, Nanolive) and a screening platform in an incubator (IncuCyte S5, Sartorius). Users are trained to work independently on the microscopes, although MHCF staff are always available for assistance.

Four high-power workstations have software for image restoration functions like deconvolution and 3D visualisation and analysis. The MHCF also develops custom solutions by macro programming in open source software (e.g. Fiji, ImageJ, or ilastik) or assembling predefined building blocks in Columbus. Most of these software tools can analyse images using machine learning and artificial intelligence.

The MHCF also provides a variety of histology techniques. In addition to semi-automated fixation and paraffin embedding, machines for sectioning paraffin-embedded, frozen, gelatine/agarose embedded or fresh tissue are available. Users can also access optimised protocols for immunodetection and tissue clearing, as well as solutions for tissue staining.

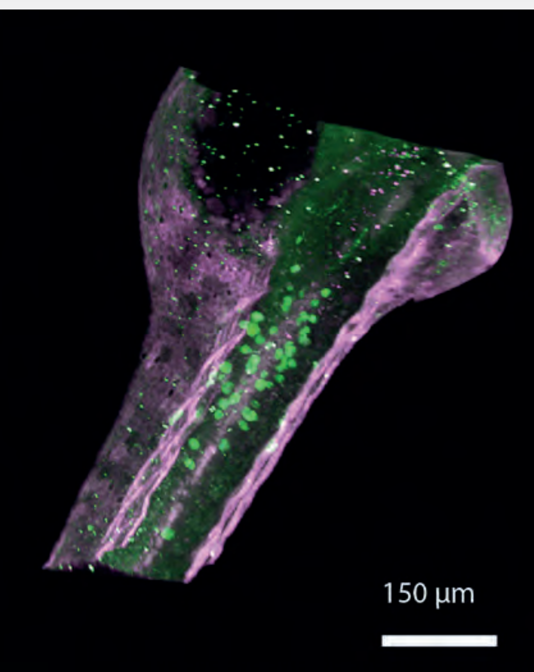

Figure 1. 3D-volume reconstruction and germ cell classification by machine learning (Imaris, Bitplane) in an albino zebrafish embryo (3 dpf) with antibody staining for germ cells (Ziwi, Alexa Fluor 555). Whole mount sample with tissue clearing (CUBIC) acquired at the spinning disk confocal microscope (VisiScope, Visitron Systems). Sample kindly provided by Nadine Wittkopp (Ketting group, IMB) and analysed by Sandra Ritz (MHCF). Video showing the sample in 3D is available at:
https://seafile.rlp.net/d/530f21adc5874606957d/files/?p=%2FZebrafish.mp4

PROTEIN PRODUCTION

OVERVIEW

The Protein Production Core Facility (PPCF) provides support with the design, expression, purification and development of assays for recombinant proteins used in IMB's research. This includes producing purified proteins for crystallography, as well as antibodies and enzymes for activity assays. The facility also offers a variety of common protein tools routinely used by IMB researchers on a day-to-day basis, such as ligases, polymerases and proteases.

SERVICES OFFERED

The PPCF supports researchers throughout the process of protein production. This includes screening suitable expression systems and vectors, optimisation of purification steps, upscaling of protein production and purification, as well as functional analysis and assay development with the purified products. The facility is equipped with four automated chromatography systems, which enable the use of the latest chromatographic methods for state-of-the-art protein purification strategies.

Another key function of the PPCF is to generate and perform functional quality control of routine laboratory enzymes and affinity probes for IMB researchers. The PPCF currently offers 29 products to IMB scientists, matching the most frequently used protein tools at the institute.

The demand by IMB's research groups for PPCF support in producing recombinant proteins and developing assays has been steadily increasing over the years. In addition to the Head, a full-time staff scientist was hired to assist researchers with their project needs and can offer services tailored to specific user requests.

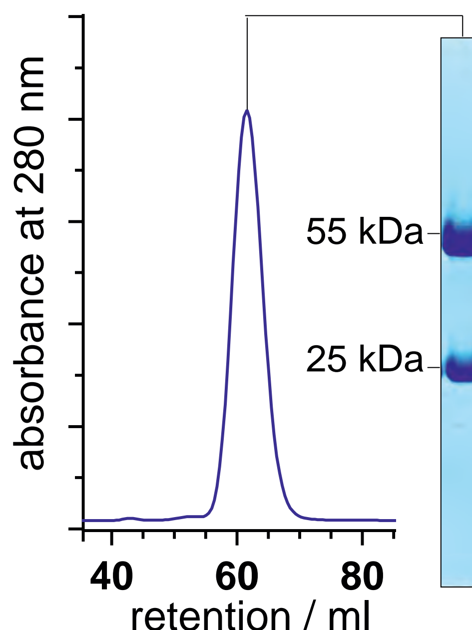

Figure 1. Elution profile of a monoclonal antibody during the final gel filtration step, showing the absorbance at 280 nm over the column volume (retention). The heavy and light chains of the antibody are visualised by Coomassie-stained SDS-PAGE next to the elution profile.

CORE FACILITY MEMBERS

Martin Möckel
Head; since 03/2018

Claire Mestdagh
Technical Assistant; 03/2020 – 08/2021

Sabine Ruegenberg
Staff Scientist; since 10/2021

PROTEOMICS

OVERVIEW

The Proteomics Core Facility (PCF) operates 30% of an EASY nLC 1000 ultraHPLC coupled online to a Q Exactive Plus mass spectrometer to perform proteomic measurements. The PCF also participates in the CRC 1361 on "Regulation of DNA Repair & Genome Stability" by providing the infrastructure for its Z3 subproject. For this project, the facility was expanded in 2019 with an Exploris 480 (Thermo Scientific) mass spectrometer and a full-time staff scientist position.

CORE FACILITY MEMBERS

Falk Butter
Head; since 05/2013

Jasmin Cartano
Technician; since 02/2014

Jiaxuan Chen
Staff Scientist; since 03/2019

Mario Dejung
Bioinformatician; since 05/2014

Amitkumar Fulzele
Staff Scientist; since 06/2020

SERVICES OFFERED

As a general service, the PCF provides band identification, analysis of posttranslational modifications on single proteins, and quantitation of labelled proteins using methods like SILAC (stable isotope labelling with amino acids in cell culture) or reductive dimethylation (DML). More advanced techniques like TMT (tandem mass tagging) for large scale quantitation of up to 10 samples in parallel or label-free quantitation can be offered in a collaborative context. In total, 1,500 measurement hours on a state-of-the-art mass spectrometry platform are provided annually to IMB and the surrounding research centres in Mainz. Mass spectrometry is provided as a full service by the PCF, including initial consultation, sample preparation and basic proteomics data analysis. Advanced proteomic workflows and in-depth statistical and bioinformatics analysis are available in a collaborative context as well. The PCF offers lectures on proteomics and data analysis and provides researchers with hands-on experience in practical courses.

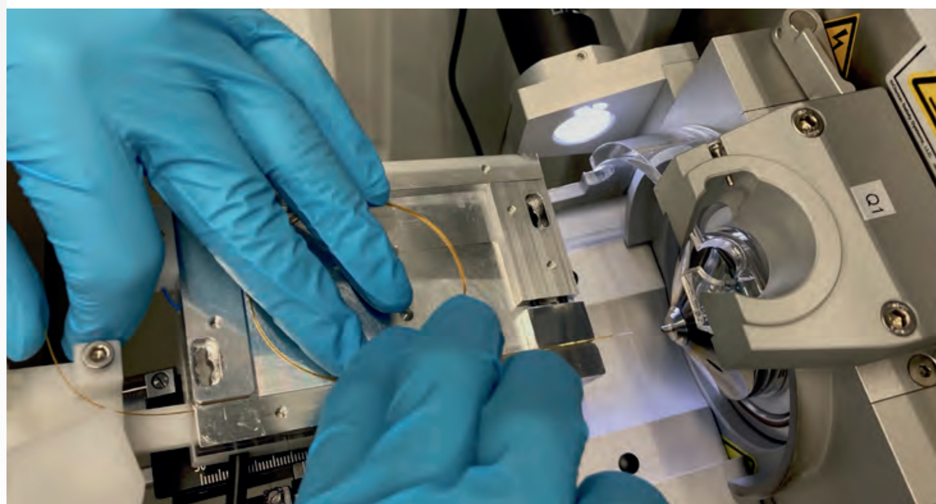

Figure 1. Mounting an analytic column in front of the mass spectrometer. The column oven improves the fluidity of the solvent and reduces the back pressure.

MEDIA LAB

OVERVIEW

The Media Lab primarily supports scientific groups and other Core Facilities by producing media, buffers and agar plates. In addition, the Media Lab is responsible for the administration of three supply centres, plasmid/cell line banks, S1/S2 waste management and the cleaning and sterilisation of glassware.

SERVICES OFFERED

The Media Lab provides the following services:

- 24/7 supply of routinely-used buffers, solutions, liquid media and agar plates for molecular biology research and for culturing bacteria, yeast, insect cells and *C. elegans*
- Production of made-to-order media
- Management of three supply centres for enzymes, kits and cell culture media
- Administration of a vector data bank, human ORF clone collection and cell line bank
- Overnight cultures for plasmid preparation
- Sterilisation of solutions/media
- Cleaning and sterilisation of glassware and lab equipment
- Autoclaving of S1/S2 waste
- Maintenance of the in-house transport system

CORE FACILITY MEMBERS

Andrea Haese-Corbit
Head; since 01/2018

Doris Beckhaus
Assistant; since 05/2011

Alwina Eirich
Assistant; since 07/2013

Pascal Hagebölling
Assistant; since 01/2015

Annette Holstein
Assistant; since 04/2012

Marion Kay
Assistant; since 04/2016

Johann Suss
Assistant; since 04/2011

Figure 1. The Media Lab produces 120L of buffers and media per week.

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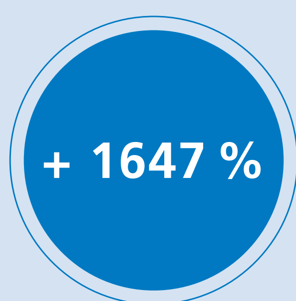
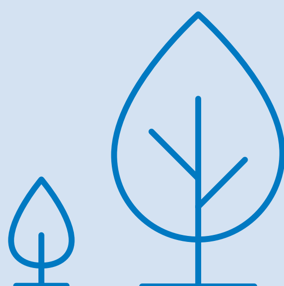
IMB STAFF

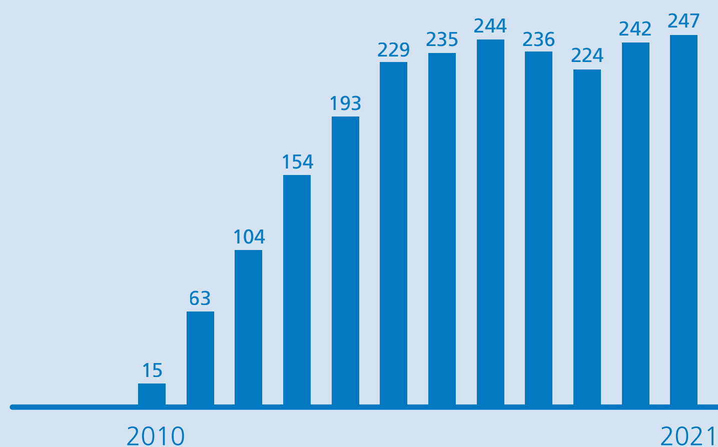
NATIONALITIES OF IMB STAFF

Rest of the world	23 %	European	77 %

IMB STAFF GROWTH SINCE 2010







EMPLOYEES BY STAFF CATEGORY

Category	Number	Category	Number
Masters & Undergraduate Students	5	Postdocs	57
Technical Staff	60	Group Leaders	19
PhD Students	79	Admin & Scientific Management	29

EXTRAMURAL FUNDING

IN ADDITION TO BOEHRINGER INGELHEIM STIFTUNG CORE FUNDING

MAJOR FUNDERS

DFG

German Research Foundation (DFG)

54.8 %

25.8 %

European Research Council

EU (ERC + EC)

RheinlandPfalz
MINISTERIUM FÜR WISSENSCHAFT UND GESUNDHEIT

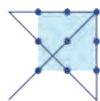
Ministry of Science and Health (MWG) of Rhineland-Palatinate

10.1 %

FURTHER SUPPORT

Leopoldina
Nationale Akademie der Wissenschaften

9.3 %



KLAUS TSCHIRA STIFTUNG GEMEINNÜTZIGE GMBH

Wilhelm Sander-Stiftung
fördert medizinische Forschung

SCIENTIFIC MANAGEMENT

We support our scientists across a range of areas so they can focus on their research

At IMB, we foster our scientists' success by providing an excellent environment for their research. This is the main responsibility of IMB's Scientific Management team.

We know that research involves a lot of time-consuming administrative work, from screening job applications, organising training for students and writing reports to managing funds and publicising key achievements. To relieve our scientists of these tasks, Scientific Management takes on such non-scientific duties, freeing up their time for quality research.

Another important way we support our scientists is to build a friendly atmosphere in which scientists enjoy working. This encourages collaborations that spark innovative ideas and build a strong community spirit. Through a wide range of scientific events, we also allow our scientists to engage with outstanding leaders in research from around the world at IMB.

Scientific Management achieves these goals through a range of services in, for example, the following areas:

+ Communications & Outreach
+ Event Management
+ Scientific Writing
+ Fundraising & Grant Management
+ Recruitment, Training & Career Development
+ Technology Transfer
+ Reporting, Research Evaluations & Strategic Planning

Ralf Dahm
Director of Scientific Management

IMB'S SCIENTIFIC MANAGEMENT HAS DEDICATED OFFICES FOR SERVICES IN:

COMMUNICATIONS & EVENTS

At IMB, we recognise that building a strong **collaborative spirit** and encouraging frequent **social interactions** between our scientists are key for productive **scientific exchange**. To facilitate this, we organise a wide range of events for our scientists to share their research with each other, with the scientific community, and with the general public. We also invite top experts from all over the world to speak about their work in Mainz. Events we organise include:

+ Seminars, conferences, workshops & symposia
+ Public outreach activities
+ Institutional reviews
+ Social events & retreats

We also maintain connections between current staff and IMB's alumni by keeping them informed of the latest developments via newsletters and IMB's social media channels.

FUNDRAISING & GRANT MANAGEMENT

Getting funding is essential for scientists to embark on ambitious projects and advance their careers. The Grants Office **maximises our scientists' chances of getting funded** by helping them find, obtain and manage extramural funding. Our services include:

+ Screening & informing scientists of calls for grants & fellowships
+ Supporting scientists in the application process
+ Administering existing project grants

SCIENCE WRITING

For junior scientists, it is extremely important that their breakthroughs are seen by the scientific community. We **increase the visibility of our scientists' work** to a broad audience by producing and distributing scientific and general texts about IMB's research. We help with writing texts for:

+ Press releases, IMB's website & social media posts
+ Annual Reports
+ Institutional grant applications

RECRUITMENT, TRAINING & CAREER DEVELOPMENT

IMB has a vibrant and international population of young scientists who are the driving force behind our exciting research. New students and postdocs are recruited to IMB through the Training Office, which also guides their career development. We work to **attract talented young researchers** and manage IMB's **structured training programmes**, ensuring that each provides a comprehensive range of courses, lectures and career events to target the specific needs of our scientists and prepare them for the next step of their career. IMB's programmes in this area include:

+ The International Summer School (ISS) for undergraduate students
+ The International PhD Programme (IPP) for graduate students
+ The IMB Postdoc Programme (IPPro) for more experienced scientists
+ The Career Development Programme for Junior Group Leaders

You can learn more about these programmes on the following pages.

As a thriving international research centre, IMB focuses on giving our researchers the best possible environment in which to do their science.

At IMB, scientists work at the forefront of their fields to answer key questions in how organisms grow, age and develop disease. Through the discoveries already made at IMB, we are beginning to transform our understanding of gene regulation, epigenetics and genome stability.

FACTS & NUMBERS

IMB's scientists produced over **348** publications in the last 5 years, with **72** in 2021 (of which **39%** were in journals with an IF of 10 or higher)

RESEARCH AND TRAINING

IMB has 3 specialised training programmes for scientists at each stage of their career:

We actively support our scientists as their careers develop by providing comprehensive training in scientific, technical and complementary skills, including:

- **Scientific & technical training** in state-of-the-art equipment by experts, as well as technical support in implementing the latest techniques
- **Professional skills training** in presentation, scientific writing, project management, fundraising, career development, negotiation and leadership by qualified trainers

Through this dedicated training, our scientists gain a competitive edge at all stages of their career in both academic and commercial settings.

IMB POSTDOC PROGRAMME

FACTS & NUMBERS

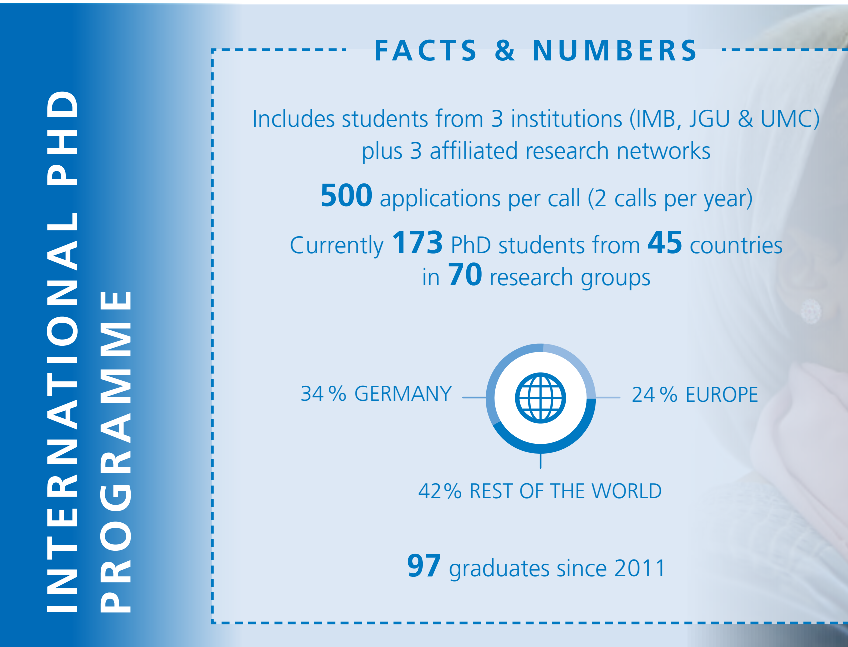
Currently **57** postdocs from **19** countries

22 % GERMANY

28 % EUROPE

50 % REST OF THE WORLD

Alumni work in industry, academia and beyond as: › Assistant Professors › Lab heads › Senior research scientists › Managers › Policy & governance officers › Consultants



INTERNATIONAL PHD PROGRAMME

FACTS & NUMBERS

Includes students from 3 institutions (IMB, JGU & UMC) plus 3 affiliated research networks

500 applications per call (2 calls per year)

Currently **173** PhD students from **45** countries in **70** research groups

34 % GERMANY

24 % EUROPE

42% REST OF THE WORLD

97 graduates since 2011

INTERNATIONAL SUMMER SCHOOL

FACTS & NUMBERS

350 applicants for **20** positions each year

154 participants from **37** countries since 2012

Rated as **"excellent"** or **"very good"** by **99 %** of participants

70 % "EXCELLENT"

29 % "VERY GOOD"

1 % "AS EXPECTED"

IMB's Postdoc Programme (IPPro) was established to meet the specific needs of our postdoctoral community. The programme provides ambitious, early-career scientists with the skills and guidance necessary to develop into future scientific leaders.

The IPPro actively supports our young professionals as their careers develop. We provide:

+ Advanced training in **scientific methods** and **professional skills** through a range of lectures, focused workshops and tailored events
+ **Guidance** from leading scientists and **mentoring** from IMB's structured Mentoring Programme for Junior Scientists
+ Networking at **career events** and **symposia** with leading external scientists from industry and academia

In a 2021 survey: **81%** of IMB postdocs felt supported by the IPPro and **70%** were satisfied or very satisfied with their training.

As well as offering fully funded positions, we also support our postdocs to raise funds for their research to help them become more independent. Collectively, the IPPro ensures that our postdocs have access to the training and technology needed to effectively carry out their research projects and advance their prospects in building successful careers.

www.imb.de/postdocs

PhD students are key to our research at IMB. To provide the structure, training and supervision necessary to excel during a PhD, IMB created the International PhD Programme (IPP) with the help of funding from the Boehringer Ingelheim Foundation.

Within this programme, our students tackle ambitious research projects, receive a broad and diverse education and have easy access to the expertise and equipment needed to drive their projects forward.

This training includes:

+ **Regular supervision** from 3 or more experts
+ Training in **scientific, professional & technical skills**
+ **Networking** opportunities at symposia, retreats & seminars

In 2021, a panel of independent reviewers representing leading PhD programmes at German and international institutions said the IPP is "**a model for a structured PhD programme, which is on par with the most prominent schools in Europe**."

With the comprehensive scientific and technical training the IPP provides, our students are prepared for successful careers in the quickly evolving field of life sciences.

www.imb.de/PhD

IMB's International Summer School (ISS) is a 6-week programme on "Gene Regulation, Epigenetics & Genome Stability" that brings talented undergraduate and Masters students from around the world to Mainz every summer. Through the ISS, enthusiastic students get the chance to work on their own hands-on project at the forefront of biological research. In 2021, the ISS was successfully organised as a 3-week online event due to the COVID-19 crisis.

The informal and international environment of the ISS gives participants an excellent framework in which to develop their practical and professional skills. This includes:

+ **Training by leading experts** in scientific and transferable skills needed as a scientist
+ **Lectures** to learn comprehensive insights into the latest research
+ **Networking** with leading international researchers

The ISS teaches students to identify key open questions in the fields of gene regulation, epigenetics & genome stability and prepares them to tackle ambitious Masters or PhD projects. This gives our ISS alumni a head start in their careers, with many going on to study and work at prestigious institutions around the world.

www.imb.de/ISS

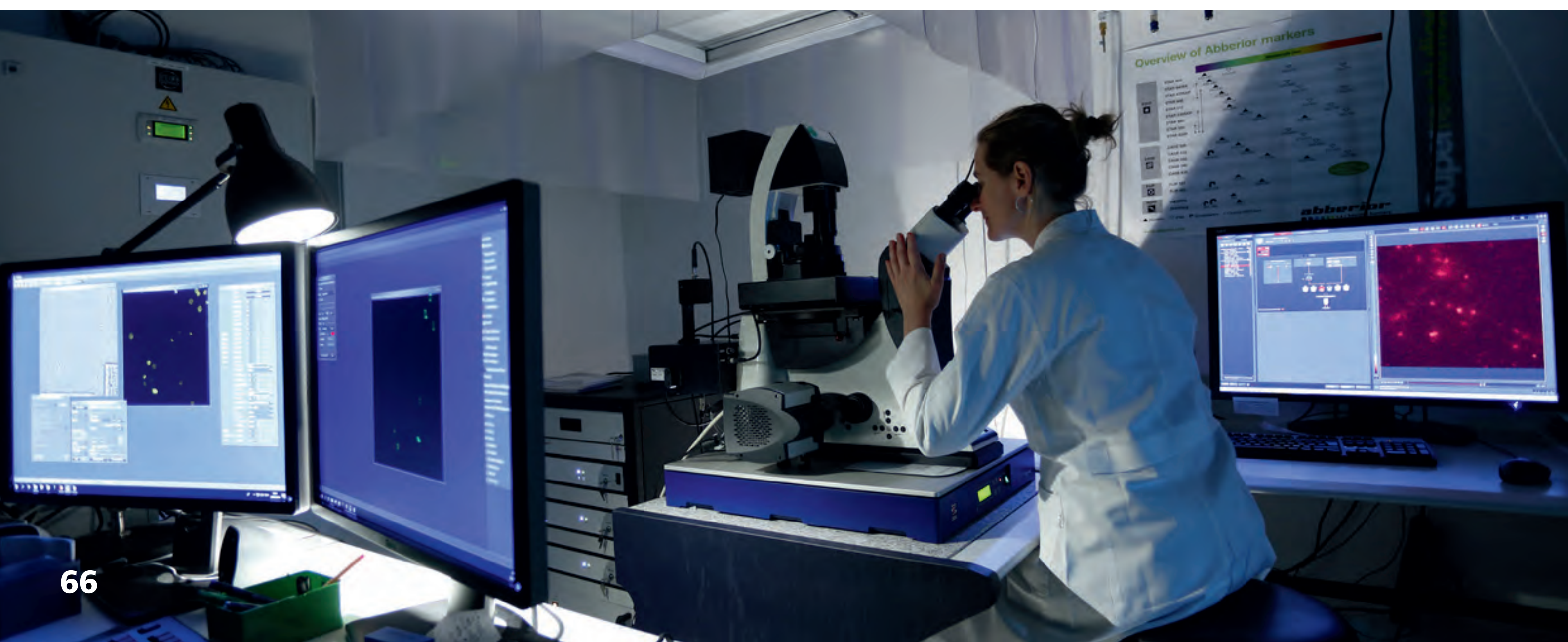
TRAINING COURSES

CORE FACILITIES TRAINING

IMB's Core Facilities staff provide our scientists with training in key scientific techniques and a wide range of cutting-edge methodologies to ensure they can consistently perform top-quality research. In 2021, IMB offered the following training courses:

LECTURES

CORE FACILITY	DATES	TITLE
GENERAL	12 Apr	Molecular & Biochemistry Techniques
BIOINFORMATICS	17 May	Databases in Bioinformatics
	14 Jun	Design & Analysis of NGS Experiments
FLOW CYTOMETRY	13 Apr	Flow Cytometry: Introduction I
	20 Apr	Flow Cytometry: Introduction II
	27 Apr	Advanced Flow Cytometry: Principles of Cell Sorting
	21 Jun	Flow Cytometry
GENOMICS	07 Jun	Genomics (NGS)
MICROSCOPY & HISTOLOGY	19 Apr	Introduction to Microscopy
	26 Apr	Microscopy: F-Techniques & Super-Resolution
	03 May	Histology & Fluorescent Labelling
	31 May	Image Manipulation: The Slippery Slope to Misconduct
	28 Jun	Electron Microscopy
PROTEOMICS	05 Jul	Proteomics
PROTEIN PRODUCTION	10 May	Protein Production & Crystallography



PRACTICAL COURSES

CORE FACILITY	DATES	TITLE
BIOINFORMATICS	07 Apr	Bioinformatics: Introduction to Biostatistics
	14 Apr	Bioinformatics: Introduction to Biostatistics
	21 Apr	Bioinformatics: Introduction to Biostatistics
	28 Apr	Bioinformatics: Introduction to Biostatistics
	03 May	Bioinformatics: Introduction to Biostatistics
	12 May	Bioinformatics: Introduction to Biostatistics
	19 May	Bioinformatics: Introduction to Biostatistics
	04 Oct	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part I)
	07 Oct	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part II)
	11 Oct	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part III)
	14 Oct	Bioinformatics: Introduction to Base R for Data Analysis & Visualisation (Part IV)
	18 Oct	Bioinformatics: Introduction to RNA-seq Analysis with Galaxy & R (Part I)
	21 Oct	Bioinformatics: Introduction to RNA-seq Analysis with Galaxy & R (Part II)
	25 Oct	Bioinformatics: Introduction to ChIP-seq Analysis with Galaxy & R (Part I)
	28 Oct	Bioinformatics: Introduction to ChIP-seq Analysis with Galaxy & R (Part II)
	05 Oct	Bioinformatics: Introduction to Biostatistics
	12 Oct	Bioinformatics: Introduction to Biostatistics
	19 Oct	Bioinformatics: Introduction to Biostatistics
	26 Oct	Bioinformatics: Introduction to Biostatistics
	02 Nov	Bioinformatics: Introduction to Biostatistics
	09 Nov	Bioinformatics: Introduction to Biostatistics
	16 Nov	Bioinformatics: Introduction to Biostatistics
FLOW CYTOMETRY	21 – 22 Apr	Flow Cytometry Practical Course
	28 – 29 Apr	Flow Cytometry Practical Course
	05 – 06 May	Advanced Flow Cytometry Practical Course: Principles of Cell Sorting
MICROSCOPY	22 – 26 Mar	Image Processing & Analysis
	13 – 15 Sep	Super-Resolution Imaging
PROTEOMICS	03 – 04 Mar	Proteomics Data Analysis
	02 – 04 Nov	Proteomics Practical Course

TRAINING COURSES

SCIENTIFIC & PROFESSIONAL SKILLS TRAINING

IMB provides our scientists with comprehensive training spanning both scientific and professional skills. This ensures they have the expertise to succeed in their careers. All courses in 2021 were offered online.

LECTURES

DATES	TITLE
13 Apr – 29 Jun	CRC 1361 Lecture Series on "DNA Repair & Genome Stability"
21 Apr	How the COVID-19 Pandemic Impacts Scientists – about risks and coping options
22 Apr	Essential Data Analysis & Reporting for Quality Manuscript Submissions
10 May	Navigating Uncertainty During COVID Times
14 – 25 Jun	Advanced Lectures on "Gene Regulation, Epigenetics & Genome Stability"
19 Jul	The Human Element: Managing mental health and why we are more than just our research
07 Aug	Looking After Your Mental Health in Academia
13 Aug	The Reproducibility Crisis: Can you trust your data?
13 Dec	Gender and Diversity Sensitive Communication in Science

PRACTICAL COURSES

DATES	TITLE	IPP/IPPro
12, 15, 19 & 22 Jan	Scientific Writing	IPP
18 – 19 Feb	True Data	IPP
02 Mar	Adobe Illustrator (Beginner Level)	IPP
04 – 05 Mar	True Data	IPP
16 Mar	Adobe Illustrator (Beginner Level)	IPP
26 Mar	Resilience in Times of the Coronavirus Pandemic	IPP
29 Mar & 12 Apr	Digital Presentations	IPP
14, 15, 22 & 23 Apr	Project Management	IPP
10 – 11 May	Critical Reasoning & Logic	IPP
17 – 18 May	Writing for the Public	IPP
25 May & 11 Oct	Stress Competence	IPP
28 May & 11 Jun	Getting Your Paper "Through the Hoops": Writing for greatest success, from submission to publication	IPP
31 May & 02 Jun	Data Visualisation for Scientists	IPPro
23 – 25 Aug	Scientific Presentations	IPP
06 Sep	Scientific Writing	IPP
07 Sep	Adobe Illustrator (Intermediate Level)	IPPro
14 & 21 Oct	Strategic Application	IPP
08 & 15 Nov	Getting Your Paper "Through the Hoops": Writing for greatest success, from submission to publication	IPP
06 Dec	Proposal Writing	IPP

INVITED SPEAKERS 2021

IMB hosts regular talks with prestigious international leaders to promote networking and exchange of novel scientific ideas. All seminars in 2021 were offered online.

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
19 Jan	IMB Seminar ◆	**Bernhard Horsthemke**	University of Duisburg-Essen, DE	Whole genome DNA methylation analysis of male germ cells in fertile, infertile and ageing men
21 Jan	IMB Green Seminar	**Nikoline Borgermann**	Ava Sustain, Copenhagen, DK	How to go green in a wet lab
28 Jan	IMB Seminar ●	**Alessandro Costa**	The Francis Crick Institute, London, UK	Eukaryotic DNA replication visualised by cryo-EM
11 Feb	IMB Seminar ●	**Susan M. Gasser**	FMI Basel, CH	Remodelling the chromatin landscape in response to damage: dynamics and degradation
18 Feb	IMB Seminar	**Peter Becker**	Biomedical Center Munich, LMU Munich, DE	Snapshots of divergent evolution towards sex chromosome-specific gene regulation
23 Feb	IMB Seminar ◆	**Yannick Wurm**	Queen Mary University London, UK	Evolutionary innovations in ants & bees: genetic architectures & constraints
25 Feb	IMB Seminar ●	**Daniel Durocher**	University of Toronto, CA	Synthetic lethality and DNA repair
11 Mar	IMB Seminar ●	**Dominik Boos**	University of Duisburg-Essen, DE	A regulation platform of vertebrate replication origin firing – the MTBP-Treslin complex
18 Mar	IMB Seminar	**Myriam Gorospe**	National Institute on Aging, NIH, Baltimore, USA	Regulatory RNA in cellular senescence
13 Apr	IMB Seminar ◆	**Jacintha Ellers**	Free University of Amsterdam, NL	Genome evolution during symbiosis: a tale of loss and expansion
15 Apr	IMB Seminar ●	**Nick Gilbert**	University of Edinburgh, UK	Regulation of large scale chromatin architecture in human cells
22 Apr	IMB Seminar ●	**Agnel Sfeir**	Sloan Kettering Institute / MSKCC, New York City, USA	DNA repair: A tale of two genomes
27 Apr	IMB Green Seminar	**Patrick Frick**	PAN-Biotech, Aidenbach, DE	Cell culture for future
29 Apr	IMB Seminar	**Jan-Michael Peters**	Research Institute of Molecular Pathology (IMP), Vienna, AT	How cohesin folds the genome by loop extrusion

● Seminars which were part of the CRC 1361 seminar series on "Regulation of DNA Repair and Genome Stability"

DATE	EVENT	SPEAKER	INSTITUTION	TITLE
06 May	IMB Seminar ●	**Dipanjan Chowdhury**	Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA	Expanding the scope of DNA repair factors – replication and beyond
12 May	IMB Seminar	**Núria López-Bigas**	Institute for Research in Biomedicine, Barcelona, ES	Tumour genomes shed light into somatic mutational processes and cancer vulnerabilities
20 May	IMB Seminar	**Rudolf Jaenisch**	Whitehead Institute for Biomedical Research, Cambridge, USA	Reverse-transcribed SARS-CoV-2 RNA can integrate into the human genome and can be transcribed in patient-derived tissues
01 Jun	IMB Seminar ◆	**Marie-Anne Félix**	Department of Biology Ecole Normale Supérieure (IBENS), Paris, FR	A model organism in the wild: *C. elegans* in an evolutionary and ecological context
21 Jun	IMB Seminar	**Brenda Schulman**	Max Planck Institute of Biochemistry, Martinsried, DE	Dynamic E3 ubiquitin ligase mechanisms
09 Aug	Ad Hoc Seminar ●	**Kyle Miller**	The University of Texas at Austin, USA	Protecting genome integrity: chromatin, cancer and the DNA damage response
16 Aug	IMB Seminar	**Alexei Aravin**	California Institute of Technology (Caltech), Pasadena, USA	Ancient Argonautes
28 Aug	IMB Seminar ◆	**Bill Wickstead**	University of Nottingham, UK	Different kit, same rules? Evolution and regulation of the non-canonical kinetochores of trypanosomes
05 Oct	IMB Seminar ◆	**David Gokhman**	Weizmann Institute of Science, Rehovot, IS	Human evolution through the lens of gene regulation
14 Oct	IMB Seminar ●	**Andrew Deans**	St Vincent's Institute, Melbourne, AU	Biochemical reconstitution of the Fanconi anaemia pathway and its role in genome stability
27 Oct	IMB Green Seminar	**Kerstin Hermuth-Kleinschmidt**	NIUB Sustainability Consulting, Freiburg, DE	The Ecomapping-method: a practical approach to make your lab more sustainable
09 Dec	IMB Seminar ●	**Johannes Walter**	Harvard Medical School, Boston, USA	Transcription-coupled DNA repair in a test tube

◆ Seminars which were part of the Research Training Group GenEvo seminar series on "Gene Regulation in Evolution: From molecular to extended phenotypes"

RESEARCH INITIATIVES

IMB is a major driver in three major research initiatives, bringing together scientists from multiple centres across Germany.

CRC 1361 REGULATION OF DNA REPAIR & GENOME STABILITY

SPOKESPERSON: HELLE ULRICH

The Collaborative Research Centre 1361 was launched in January 2019 and is funded by the DFG with €12.4 million until December 2022. This initiative consolidates 18 projects from investigators across 7 institutions (IMB, JGU, UMC, Darmstadt University, Munich University, Frankfurt University and Jena University) with the goal of understanding the molecular mechanisms modulating the activities of genome maintenance in the cell.

Supported by 3 dedicated service projects and centralised management, the network comprises experts in structural biology, organic chemistry, biochemistry, molecular & cell biology, genetic toxicology and clinical sciences. The CRC's Integrated Research Training Group is designed to ensure that participating students receive the best possible training and career development while completing their PhDs.

In 2021, researchers in the CRC 1361 published a total of 27 papers that were directly connected to projects in this initiative and 3 new associated members joined. A highlight was the CRC 1361 Autumn Meeting, which took place on-site in the MEWA Arena Mainz in September and offered researchers an opportunity to present their work and network with other groups in the initiative.

www.sfb1361.de

RTG GENEVO: GENE REGULATION IN EVOLUTION

SPOKESPERSONS: SUSANNE FOITZIK (JGU) & RENÉ KETTING (IMB)

The Research Training Group GenEvo launched in June 2019 and is funded by the DFG with €5.8 million until December 2023. This initiative is a collaboration between IMB and JGU's Faculty of Biology and is centred around the core question of how complex and multi-layered gene regulatory systems have both evolved and driven evolution. Mixing both junior and senior researchers, GenEvo brings together 12 outstanding scientists in 14 projects, fusing expertise in evolutionary and molecular biology. The programme focuses on training a new generation of PhD students to work on ambitious research projects at the interface of these two themes, while also receiving a broad, interdisciplinary education.

In 2021, researchers in GenEvo published a total of 5 papers that were directly connected to projects in this initiative and 3 new group leaders joined as contributing members. A highlight was the GenEvo Mid-term event, which took place online in May and hosted international experts in the field of evolutionary and molecular biology. GenEvo PhD students also had the chance to network in-person with PhD students from the RTG EvoPAD at the University of Münster during a joint summer school in September. Finally, in November GenEvo group leaders strengthened their collaborations by presenting their current results and project proposals for the next PhD student cohort at the GenEvo group leader retreat at Ebernburg, Bad Kreuznach.

www.imb.de/genevo

SCIENCE OF HEALTHY AGEING RESEARCH PROGRAMME (SHARP)

STEERING COMMITTEE MEMBERS: PETER BAUMANN (IMB/JGU), STEPHAN GRABBE (UMC), CHRISTOF NIEHRS (IMB) & HANSJÖRG SCHILD (UMC)

In June 2021, IMB launched SHARP, a joint PhD training programme with Mainz University and the University Medical Center Mainz (UMC). The goal of SHARP is to strengthen cooperation between research groups at IMB and UMC in projects focusing on ageing and longevity. This programme brings together 25 group leaders, including clinical/preclinical researchers from the fields of stem cell biology, epidemiology, immunology, cardiology, neurobiology and cancer biology together with basic molecular biology researchers in the fields of epigenetics, proteomics, telomere biology, RNA biology, DNA repair and autophagy.

By combining the complementary skills of basic and clinical/translational researchers, SHARP will gain new insights into the underlying causes of ageing and age-related diseases and discover new ways to successfully prevent them. SHARP is funded by Rhineland-Palatinate's Ministry of Science, Education and Culture with €1.8m from 2021-2024. The first SHARP PhD students began their studies in September 2021.

www.cha-mainz.de/SHARP

SCIENTIFIC EVENTS

Scientific events organised by IMB in 2021 include:

4–6 May

GENEVO MIDTERM EVENT

"GENE REGULATION IN EVOLUTION"

Scientific organisers: Susanne Foitzik (JGU), René Ketting (IMB)

30 June–1 July

WORKSHOP ON "EPIGENETICS OF AGEING"

VIRTUAL TEASER EVENT FOR THE 2022 IMB CONFERENCE ON "EPIGENETICS OF AGEING: RESPONSES TO ADVERSITY ACROSS SCALES"

Scientific organisers: Joan Barau (IMB), Peter Baumann (IMB/JGU), René Ketting (IMB), Beat Lutz (JGU), Meng Wang (Baylor College of Medicine, Houston)

28 September

SFB 1361 AUTUMN MEETING ON "REGULATION OF DNA REPAIR AND GENOME STABILITY"

Scientific organiser: Helle Ulrich (IMB)

SCIENTIFIC ADVISORY BOARD

IMB is grateful to the members of our Scientific Advisory Board for the insight, guidance and advice that they have provided in order to help us continue to be a leading research centre.

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BARAU

Mosler T, Conte F, Longo GMC, Mikicic I, Kreim N, Möckel MM, Petrosino G, Flach J, Barau J, Luke B, Roukos V and Beli P (2021) R-loop proximity proteomics identifies a role of DDX41 in transcription-associated genomic instability. *Nat Commun*, 12:7314

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AWARDS IN 2021

DOROTHEE DORMANN
Alzheimer Research Award 2021 (Hans and Ilse Breuer Foundation)

GAURAV JOSHI
PhD Student, Niehrs group. Boehringer Ingelheim Foundation (BIF) Fellowship

AGATA KALITA
PhD Student, Keller Valsecchi group. Boehringer Ingelheim Foundation (BIF) Fellowship

EDWARD LEMKE
"Life?" grant (Volkswagen Foundation)

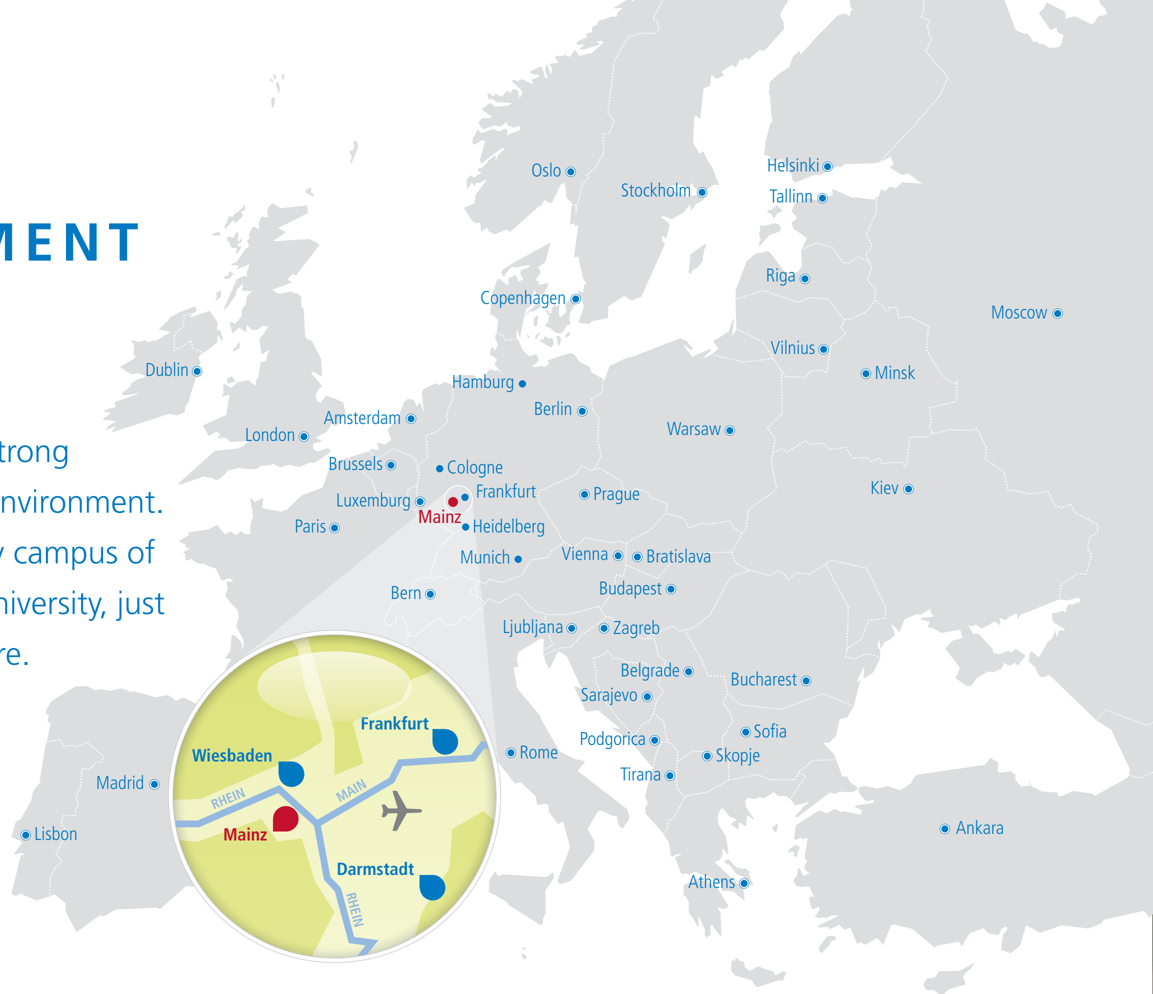
CHRISTOPHER REINKEMEIER
PhD Student, Lemke group. Karl Lohman Award (German Society for Biochemistry and Molecular Biology)
and
Rainer Rudolph Award (Rainer Rudolph Foundation)

ERIN STERNBURG
Postdoc, Dormann group. Postdoctoral Fellowship (Peter and Traudl Engelhorn Foundation)

HELLE ULRICH
Elected to the German National Academy of Sciences Leopoldina

RESEARCH ENVIRONMENT

IMB is embedded in a strong and dynamic research environment. It is located on the leafy campus of Johannes Gutenberg University, just west of Mainz city centre.



With 10 departments, more than 150 institutes and 32,000 students, Johannes Gutenberg University is one of the largest German universities. In biomedical research, the university has built strong, interdisciplinary centres dedicated to neuroscience, cardiovascular medicine, immunology and oncology.

The University Medical Centre, which is located near the main university campus, has a strong focus on clinical and translational research and has researchers who also work in close contact with IMB. In addition to the University, IMB has two Max Planck Institutes (the Max Planck Institute for Chemistry and the Max Planck Institute for Polymer Research), the Leibniz Institute for Resilience Research and Mainz's University of Applied Sciences as immediate neighbours.

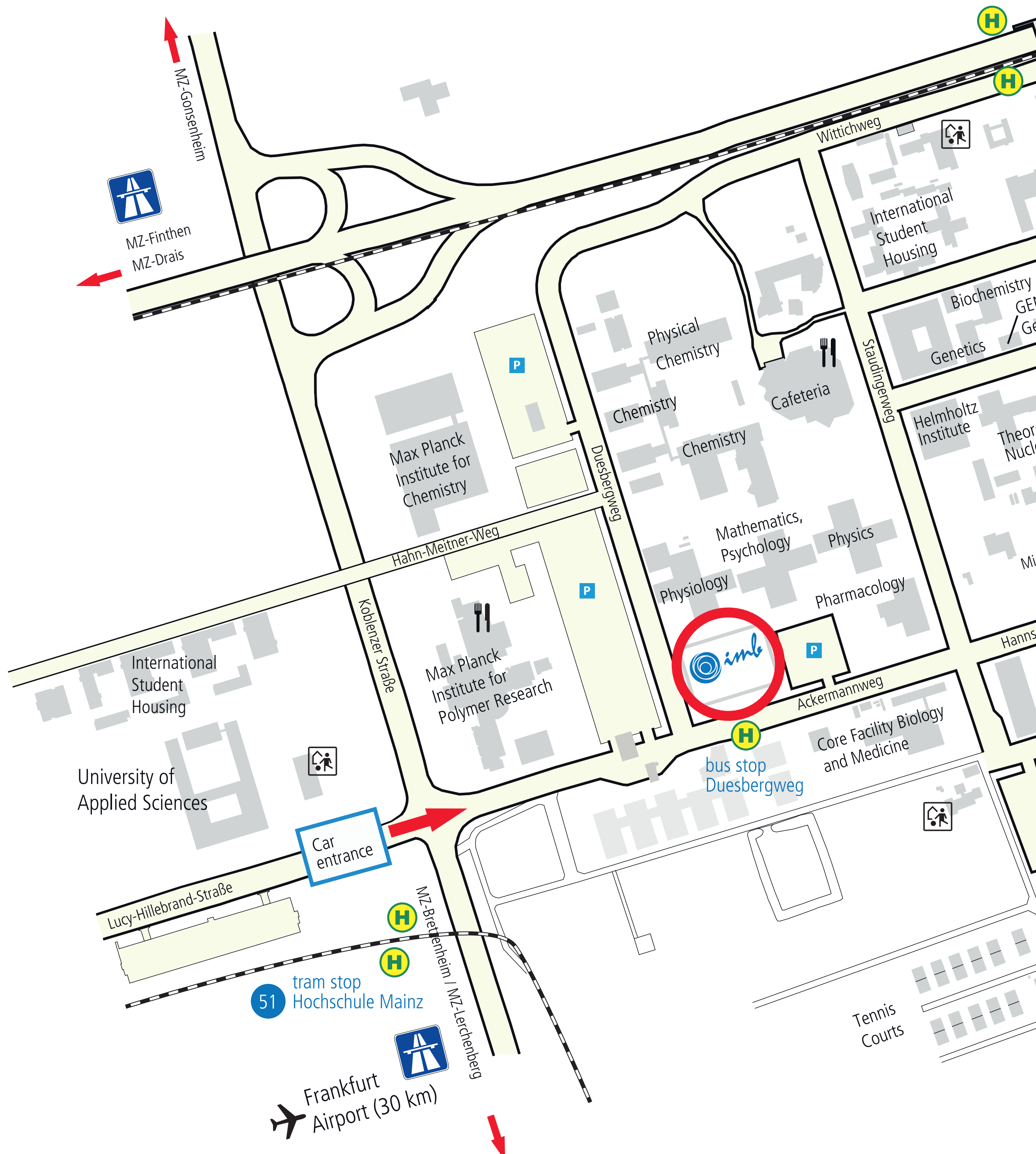
Mainz is also surrounded by a number of towns and cities with extensive research activities. For instance, Frankfurt is only 35 km away and is home to Goethe University, which has over 46,000 students and 10 research institutes within the Biochemistry, Chemistry and Pharmacy Department alone. Furthermore, there are several Max Planck Institutes in Frankfurt (including the Max Planck Institute for Biophysics, the Max Planck Institute for Brain Research and the Ernst Strungmann Institute for Cognitive Brain Research). In addition to Frankfurt, nearby Darmstadt is home to both a Technical University, whose Department of Biology has a focus on synthetic biology and the biology of stress responses, and a University of Applied Sciences that includes a focus on biotechnology.

In addition, there is an extensive industry R&D presence, with, for example, the headquarters of Boehringer Ingelheim, BioNTech, Translational Oncology (TRON) and the Merck Group in close vicinity.

WHERE WE ARE

IMB is located in Mainz, a charming, open-minded city that dates back 2,000 years to Roman times and still has a historic centre with a magnificent medieval cathedral. It was also here, in 1450, that Johannes Gutenberg invented modern book printing. The city is located at the confluence of two of the most important rivers in Germany, the Rhine and the Main, and has spectacular esplanades. Mainz is within easy reach of both cosmopolitan Frankfurt, with its famous opera house, avant-garde museums and glass-and-steel banking district, and the Rhine valley region with its castles, vineyards and nature reserves that offer great outdoor activities. With Frankfurt airport – one of the largest airports in Europe – only 25 minutes away, countless European and overseas destinations are within easy reach.

CAMPUS MAP AND CONTACT



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Johannes-v.-Müller-Weg
University Administration
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(p6) Cell phenotype detection and cortical distance in mouse brains. Image credit: Jennifer Sitta (Lutz group, University Medical Center Mainz).

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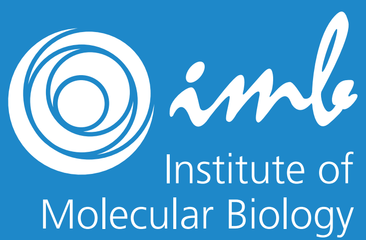

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